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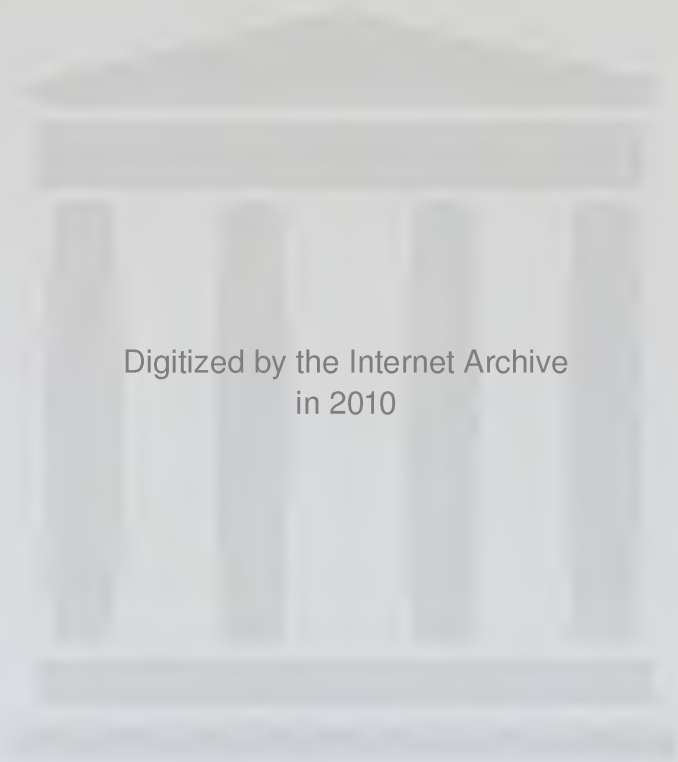
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Worker "fishing" for microbes through a microscope from a colony growth on transparent nutrient-jelly in glass Petri dish

# WHO'S WHO AMONG THE MICROBES

BY

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AND

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ILLUSTRATED

THE CENTURY CO.

NEW YORK LONDON

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*First printing*

Printed in U. S. A.

TO

T. MITCHELL PRUDDEN

# PREFACE

The following sketches grew out of a series of radio talks on communicable diseases and their microbes. The increasing demand for information suggested that people were deeply interested in these invisible, omnipresent enemies and friends. The thought occurred to The Century Co. that the information given in these talks might be more useful in a permanent shape. We were consulted with the result that the subject matter was elaborated to form this small volume.

The question of the relationship of microbes to man's welfare is so vitally important and many sided that any effort made to show some of its sides seems worth while.

We have endeavored to described simply and accurately the most important facts known that help us determine how and why some microbes are harmful to man, others harmless and still others helpful. We also tell how man can use available knowledge to protect himself against harmful ones and utilize more fully the activities of the useful.

We wish to express our great appreciation of the kindly aid of Anna I. and Harriet von Sholly in reading the manuscript and of Amelia R. Wilson in its preliminary critical typing.

The Authors.

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# WHO'S WHO
# AMONG THE MICROBES

## CHAPTER I

## EARLY DISCOVERIES

Menace of the unknown—Invisible forces—Fighting in the dark—Glimmerings of light—Revelations of the first lenses—Whence?—How?—Why?

THE world of microbes, that world of minutest living beings, had existed, invisible and unknown to man, throughout the ages. Only comparatively recently have its ever-present myriads been forced upon our attention. As late as the middle of the nineteenth century disease and death brought about by germs remained a mystery—due, believed the many, to malignant spirits. Only with the development of the microscope was it possible for man to be certain of the presence of such tiny creatures and to begin intelligently his battle to study and control them.

It is true that even as early as 40 B.C. an occasional real thinker surmised that minute organisms which the eye cannot see enter the body and cause disease.[1] And a few investigators of pre-microscope days went so far as to say that certain diseases were probably caused by a *contagium-vivium*—a living virus or poison. But the majority of people in those days still thought that the mystery of disease must be referred to gods or devils either directly or through magic or

[1] Osler, "The Evolution of Modern Medicine." New Haven: Yale University Press, 1923.

witchery. And to what lengths their obsession led them for many years we all know not only from the customs of ignorant savages but from the tragic history of more intelligent people. Witchcraft in Salem and elsewhere may be recalled as one of the many horrible situations resulting from the ignorance of fanatics.

This belief that disease was due to some malign influence that used an agency often belonging to the invisible world was true in a sense other than these bewildered people realized. The idea that disease was sometimes sent as a punishment or corrective by the gods went along with other early crude imaginings. Little wonder that people prayed and made sacrifices to their gods to avert the pestilences that descended upon them and the dread diseases that stayed with them they knew not why except it might be for their sins known or unknown. And the conviction that the gods in their wise providence sent or allowed these terrors as punishment became firmly established. Men exercised their ingenuity in devising ways of propitiating the angry gods. In very early times feasts and dances were given to appease divine wrath. Later, fasts with sackcloth and ashes were resorted to in endeavors to obtain the favor of the offended deity.

In this day of our knowledge of the prevention of infectious diseases we can scarcely realize the state of mind of people who constantly faced the menace of the unknown in the fear of being attacked by such terrible epidemic scourges as the black death (bubonic plague), yellow fever, cholera, smallpox, and the endemic or ever-present diseases of tuberculosis, syphilis and malaria, without knowing anything about their cause, prevention and cure. And in addition they saw their

crops destroyed by blights and their flocks swept away by still other mysterious diseases.

Defoe's famous "Plague Year" (1665) gives a minute vivid picture of the fearful ravages from plague in those times and of the horrible way the authorities, in their ignorance, handled the victims. One in every six or seven persons died, and the dead, and sometimes the dying, were thrown together into large pits. It is true they were covered with quicklime, which is a good germicide. Now with our knowledge of the nature of plague and its spread we have demonstrated one of the most clear-cut relationships between cause and prevention, and have shown the satisfactory results of preventive treatment based upon this knowledge in the areas where it is possible to carry out these measures. Thus in the United States there is now only an occasional sporadic case started by infection brought in from outside, though earlier a small epidemic of plague developed even here before we had learned how comparatively easy it is to prevent its spread.

Copeman [2] dramatically describes the early history of the terrors from smallpox, perhaps the most dreaded of all the formerly uncontrolled scourges, because it had become endemic, always present. Not only did it frequently cause death, but, what might be worse, it often destroyed the beauty, the physical personality even, of the survivors, "turning the babe into a changeling at which the mother shuddered, and making the eyes and cheeks of the betrothed maiden objects of horror to the lover." [3]

[2] S. Moncton Copeman, "Vaccination." London: Macmillan & Co., 1899.

[3] Macaulay's "History of England." Account of the year 1694.

These shocking conditions were due chiefly to the fact that people knew nothing about the activities of these microscopic or ultra-microscopic organisms in disease and health.

The truth only commenced to be discovered, and then slowly, when the microscope began to reveal its wonders. Even when the stupendous discovery was made that minute forms of life too small to be seen with the naked eye were swarming in myriads throughout nature, in air, water, soil, observers did not at first connect these tiny creatures with the cause of definite diseases. For they were primarily found by van Leeuwenhoek, the first of de Kruif's magnetic microbe hunters, in quite harmless vehicles, such as rain-water, the top layers of the soil and in healthy human excretions.

When these minute germs were first seen swimming around in fluids as fish do, their discoverers thought that they had found some new little animals—animalcules; so they searched for their heads, eyes, tails and other parts similar to those belonging to the larger known animals. When they could not find these structures, the hunters thought it was due to the fact that their lenses did not magnify enough, and many were the attempts to make better lenses.

Now we know that the majority of the forms they first saw were the very simplest of living creatures, for the most part with no clearly discernible structure; that is, they were motile bacteria belonging to the vegetable kingdom. Only a small number were animals, and even these were single-celled with simply a few differentiated parts called organelles.

While it was exciting enough to watch through the lenses these little creatures girate around with all sorts

of fantastic motions, the thoughts as to what they might mean, where they came from, how they live were far more alluring to the few who knew about this great discovery and busied themselves with attempts to find out all about these mysterious forms of life.

First the searchers asked themselves, "Where did these minute living forms come from?" "How were they created?" The majority thought that the answer to these questions was that these creatures were continually being generated spontaneously under certain conditions of heat, moisture and food; and many were the grotesque ideas these observers had of just how this generation took place.

The few most thoughtful investigators believed that these lowly organisms grew from preëxisting like organisms and that their beginnings lay, as did that of the beginning of all life, in the dim unknown past, or that they were created "in the beginning by God."

The many who believed that all germs continued spontaneously to generate were superficial observers, who thought in ruts. The occasional ones like Redi (1688) or Spallanzani (1769), who believed the opposite, were not only close observers but showed that they were right by actually doing the things they claimed could be done, in such a logical way and so fully controlled against error that nearly everybody with at least average intelligence who became acquainted with their work, even some of the most doubtful, were finally convinced that under known conditions germs did not spontaneously generate. The story of their convincing demonstrations has often been told, but it cannot be too often repeated, because it is such a good illustration of wrong deductions from insufficient checks or controls

against error, and right conclusions from sufficient evidence properly controlled.

The sponsors of spontaneous generation said that if one boiled broth made from vegetable or animal matters the germs that might be present would certainly be killed, and yet if such broth was kept from the air by corking the bottles containing it, in a few days it would be swarming with germs. Spallanzani decided that these broths had not been cooked long enough to kill the most resistant germs or that it had not been properly corked. So he made a large quantity of broth and put some into each of many glass flasks. Then after closing some of the flasks by fusing with heat, and others by corking with imperfectly fitted porous corks, he kept some in boiling water for a few minutes and others for at least one hour. After removing the flasks from the water he kept them under observation for some time. At length he saw that in all flasks that had been in boiling water for only a short time the broth had become turbid, due to the growth of germs, and that of those that had been in the boiling water for about an hour the fused flasks remained free from germs, while a number of the imperfectly corked ones showed them.

His opponents answered him by claiming that the boiling for so long a time without access to any air had destroyed the power of the broth to generate germs spontaneously.

Spallanzani soon showed that this was not true by the simple process of exposing some of the boiled broth in the fused flasks to the air, when the broth soon became filled with growing germs. He said that germs must produce seeds that are very resistant to heat. He thus concluded before they were seen that these tiny

creatures could develop still more minute parts like seeds which would contain the germ of the individual. Pasteur, much later, showed that the reason why some fluids required so much boiling was because of the presence of these very resistant seeds or spores which had just been demonstrated by Perty (1852).

Pasteur's clear-cut experiments showed that no life occurred in any fluids, however rich the mixture, if such fluids were boiled sufficiently and kept protected from unfiltered air or other source of contamination. These investigations of his, his telling demonstrations and the heated arguments of his opponents lasted over four years. They are vividly told in Vallery-Radot's [4] interesting "Life of Pasteur."

Finally, after more than a century of controversy, nearly every one agreed that these minute creatures did not suddenly come into life under any of the conditions observed.

One of the early practical results of this study on spontaneous generation—one of far-reaching importance—was the introduction by Appert in 1804 of the preserving of foods by sterilizing them in cans.

With this greatly contested point of spontaneous generation apparently settled, there was more time to study other questions concerning these little creatures; but with all their endeavors, students did not get very far in learning how the germs lived and what they mean, because nobody had yet learned how to separate them easily one from the other so that each kind might be grown by itself in pure cultures. That came much later. In the meantime, Ehrenberg (1838) and others

[4] Translated by Mrs. R. L. Devonshire. New York: McClure, Phillips & Co., 1902.

gave rough classifications of these minute forms based upon physical appearance or morphology alone.

During all this time there slowly multiplied the number of observers who claimed that some of these germs were probably the cause of certain diseases, and, as usual, one or two were far ahead of others in their powers to observe and deduce. First came Boyle just about the time of Kircher's and Leeuwenhoek's discoveries of germs (1659-1675), who said that infectious diseases were probably due to fermentation caused by animalcules. Then Lancisi (1718) inferred that malaria was due to an animalcule. In 1753 Linnæus emphatically stated that all disease was due to fermentation and putrefaction from an unknown living cause which he called chaos. And Plenciz (1762), a Viennese physician, published a more clear-cut statement. He maintained that all infectious diseases must be caused by micro-organisms, and each disease probably has its own kind of germ. He gave as his reasons for this belief the mode of infection, the unlimited development among large numbers of individuals with the gradual spread over wide areas, the incubation, course and resulting immunity.

It had already been shown by the Chinese and the Hindus that the inoculation of a susceptible person with smallpox virus taken from a person suffering from smallpox (which method was introduced into England by Lady Montague in 1721) usually reproduced the disease in a light form and caused immunity. Then in 1796 Jenner made his world-famous demonstrations with cowpox virus. He was led to make his first trials of it because of the oft-reiterated opinion of dairy

people that milkmaids, after developing cowpox contracted while milking, did not get smallpox after exposure to it. He showed that when inoculated into the skin of human beings, this cowpox virus produced a localized pustule resembling those of smallpox, but not spreading, and that, marvelous to relate, after this healed, the individual was subsequently protected from infection with smallpox virus. Thus he proved that the dairy people had been right in their observation.

That was indeed a wonderful discovery. Of course, there were detractors and still are. That some parts of the world are free from smallpox to-day because of wide-spread and thorough vaccinations these detractors will not accept. They claim there would be no smallpox if there were no vaccination. They have only to watch reports of outbreaks of smallpox and study the cases minutely to become convinced of the efficacy of thorough vaccination. These carpers should learn, since they apparently don't know, that each individual responds to vaccination in a different degree, so that in some the immunity lasts only one year, in others longer and in many for life; while there are even a very few that do not make immune antibodies, so there are exceptional cases that appear to receive no benefit from vaccination.

The next virus that was shown to produce a distinct disease was the rabies virus (1813). The saliva of rabid animals was shown to have the power of producing rabies when inoculated into well animals. It had been observed much earlier that man became "mad" after the bite of a rabid animal. Much later, Pasteur did his monumental work on the nature of the rabies virus and

showed that a vaccine could be made that would keep the vast majority of those bitten from developing rabies.

Up to the middle of the last century certain medical people continued to assert that the living nature of contagion was too absurd an idea to waste time in refuting, but enthusiasts on the other side studied more than ever to find evidence pointing to the truth of the idea that germs are the cause of infectious diseases.

Henle in 1840 enunciated practically all of the points quoted later as Koch's postulates. He said that if a given germ causes a given disease, it should always be found in that disease and in no other, and the tissues containing it should produce the disease when inoculated into other animals. When, finally, Pollender in 1849 actually demonstrated bacterial rods in the blood of animals dying of anthrax, and Davaine (1850) proved that only blood that contained these rods produced the disease when inoculated into healthy sheep, many investigators were ready to go to the limit in believing that all diseases are caused by germs and that all germs are dangerous.

Later discoveries proved, of course, that such was an extreme view. It is interesting to note as we scan the history of medicine the fluctuating curve of emphasis placed by different writers upon the importance of microbes to man. First germs were ignored or considered merely a curiosity. We still have ignorers. Then when dangerous germs were demonstrated, all germs were damned; some have been praised and others damned irregularly since then.

Even to-day two popular books illustrate the fact that we still have with us those who emphasize the

extremes of both sides. In one, entitled "The Conquest of Disease," [5] the statement is emphatically made that all germs are harmful to humans. On the other hand, the book "Civilization and the Microbe" [6] states very clearly and convincingly that the great majority of these minute forms are extremely useful directly or indirectly to civilized man. Many instances are given of their direct as well as indirect use to man. To believe that all are dangerous is the depth of pessimism; on the other hand, to believe that all may become useful or rendered innocuous within an appreciable time is the height of optimism. Both appear to be unwarranted by the known facts.

Notwithstanding their dread importance as disease producers and their helpful importance in breaking down complex dead materials into simple compounds for plant use and so enable life to continue, microbes are still ignored by the majority of people. Many people don't want to be bothered with the thought of all of the difficult problems concerning the relationship of these minute forms to our welfare. But to those people in whom the quality of curiosity, of wondering over the mysteries of our beginnings and our endings, is well developed, the fact that we know so little about germs is all the more reason for being interested in learning more about them. And when these eager searchers think of the many minute forms that may still be undiscovered because there is no way yet known to demonstrate them, and that while some of these germs may be harmful, some may be useful, and some

[5] David Masters, "The Conquest of Disease." New York: Dodd, Mead & Co., 1925.

[6] Arthur I. Kendall, "Civilization and the Microbe." Houghton, Mifflin Company, 1923.

of the many harmless ones may be made useful, then is their eagerness to learn about all these forms greatly enhanced.

Fortunately for our welfare, there have always been a few enthusiasts who have continued to study these elusive protista, or first forms of life, and so we have gradually come to know many of their characteristics.

An important characteristic to which we have already alluded is the formation of resistant spores or seeds by some varieties. Perty, in 1852, was the first to observe them. Others also saw them. Then Pasteur, as we have said, showed that the presence of spores explained why we still have a growth of germs after they have been submitted to a boiling temperature for a short time.

A big discovery made by Perkins (1856) helped materially in the study of these forms. Perkins discovered accidentally the value of aniline in dyes. This led to the brilliant work of Weigert (1871) and of Ehrlich (1887) on the use of these dyes in staining microorganisms, and later still to the work of Churchill and others in the use of the dyes in differential cultural methods and in treatment of certain infectious diseases.

Then the discovery of how to get pure cultures of these germs was of inestimable importance in studying them. The first method devised by Lister (1858), Pasteur and others was a dilution method. This method required great patience and an almost innumerable number of dilutions in order to catch a single organism. It is still used to obtain pure cultures of certain protozoa, but it is now seldom employed for that purpose in studying bacteria. In 1871 Klebs used hen's

eggs as a culture medium. At the same time Koch developed a jelly medium in which he obtained isolated colony growth. Then came Hesse (1881) with her agar-agar medium, which Koch later substituted so successfully for the gelatine medium.

Just at this time, when it was demonstrated that the suppuration of wounds was due to bacteria, the great English surgeon Lister, incited by Pasteur's work, introduced the use of antiseptics in his surgical operations. His work was epoch-making in its saving of life. Thus was stopped the formation of pus, even laudable pus (really praiseworthy pus it was considered then), as the small amount was called that was wrongly thought necessary for the quick healing of wounds. While later it was learned that the use of chemical antiseptics in surgery could be largely replaced by heat sterilization of apparatus and by the surgical cleaning of the hands of the operator and by the wearing of a mask, and so on, still, Lister's work was an immense advance over old methods either of letting "nature take its course" or of pouring hot oil on the wounds or using other barbarous procedures. It is true that long before Lister's time Oliver Wendell Holmes, in America (1843), maintained that puerperal fever (childbed fever), that former bane of childbirth, was due to a virus that might be carried by attendants, physicians as well as nurses, from patient to patient. And while he was ridiculed by his colleagues as an immature raver about a condition that they declared was plainly due to "Providence," he maintained his contention. He was soon brilliantly corroborated by Semmelweiss in Austria (1847), who showed that if examiners in a lying-in ward first washed their hands in a chloride of lime

solution, puerperal fever practically stopped in that ward. Of course, we know now that the disinfecting of hands, while perhaps the chief means of preventing puerperal fever and a wonderful advance over the old custom of merely washing the hands, as was ordinarily done, even though coming from the dissecting room to the wards, does not wholly prevent the danger from the carriers, which we will tell about later.

This skeptical reaction of many of the medical profession to the brave and logical presentations of Holmes and Semmelweiss illustrates again how difficult it seems to be for the majority to break away from custom. Most of the doctors strongly opposed the communicable nature theory of disease, and it was not until Lister's time that it was generally accepted.

From the discoveries in rapid succession of the specific microbes of many of the infectious diseases, and the demonstration by Pasteur that other vaccines than that against smallpox might prevent each its own disease, may be dated the beginning of the fight against dangerous microbes which constitutes a large part of modern preventive medicine. Facts in regard to these later discoveries will be given in the following chapters.

## CHAPTER II

## HOW MICROBES BECOME BETTER KNOWN

Through the microscope—By the use of stains—By the use of culture media.

Our chief aid in becoming acquainted with microbes must be, of course, the microscope. The revelations through the first lenses spoken of in our previous chapter gave only a glimpse of the subvisible world that we know to-day. Through the series of lenses in the compound microscope of to-day the lower limits of vision are extended about twentyfold over those early lenses. That is, we may see germs magnified as much as 2000 diameters, which allows us to distinguish some individual characteristics of all but a few of even these minute beings.

This wonder, the modern microscope, has been gradually evolved from the imperfectly ground low magnification lenses of Jansen in 1590, Kircher in 1659 and Leeuwenhoek in 1675. While Leeuwenhoek's lenses were an improvement over the others, their power to magnify was not much greater and they were still full of imperfections that often caused the objects studied to appear like distorted and indefinite dots and lines. One has only to visit the Army Medical Museum in Washington and view their wonderful and fantastic collection of old microscopes, one of Jansen's time and a number dating before the time of van Leeuwenhoek,

to realize what improvements have been made. With the development much later of the Huyghenian compensation eyepieces, the immersion lens, the Abbe substage condenser with its iris blender, and the series of finely ground compensation lenses of special glass in the objectives and eyepieces, our microscopes of to-day allow us to perceive more or less clear-cut pictures of all of the known microbes.

A bifocal or binocular microscope adds greatly to the comfort of the observer. Then there is the dark field microscope that was invented to help study very minute particles and so-called ultra-microscopic forms. These little points of matter catch the oblique rays of light and reflect them to the eye, thus standing out on an otherwise dark field.

In order to understand fully the structure and workings of our microscopes, a special book on the subject should be consulted.[1]

We may study individual germs through the microscope in either the living or dead condition. Since through our high-power lenses these minute organisms when living appear for the most part only as tiny bits of translucent jelly-like substances containing one or more refractile granules, they must be killed, fixed to a glass slide and stained in certain ways in order to learn more details of their structural differences and of their individual reactions to special stains.

Living, the microbes are examined in what is called a hanging drop of a fluid menstrum, or they may be planted on a hanging mass of a transparent solid medium. The hanging drop or mass is placed on the

[1] One of the good ones is Gage's "The Microscope." Ithaca, N. Y.: The Comstock Publishing Company, 1925.

center of a little square piece of thin glass (cover glass) and then inverted over a glass slide at the place where there is a hollow of about a half inch diameter. The surface of the glass around the hollow is smeared with a little vaseline or other inert oil. This allows the cover glass to stick after being pressed down and prevents the drying of the drop. In this way we can study the individual form of the microbes, also their motion, size, shape, growth, division, spore formation, vital staining, and their reactions to specific serums, such as the Widal reaction (the agglutination test) and other so-called serologic tests which we will explain later.

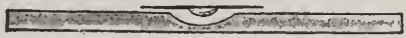


*Fig. 1*

HOLLOW SLIDE WITH COVER-GLASS FOR STUDYING A HANGING DROP OF GERMS THROUGH THE MICROSCOPE.

To examine the organisms dead they must be spread in an even film on a glass slide, dried, fixed usually by heat or alcohol and finally stained. In tissues the organisms must be examined stained in sections.

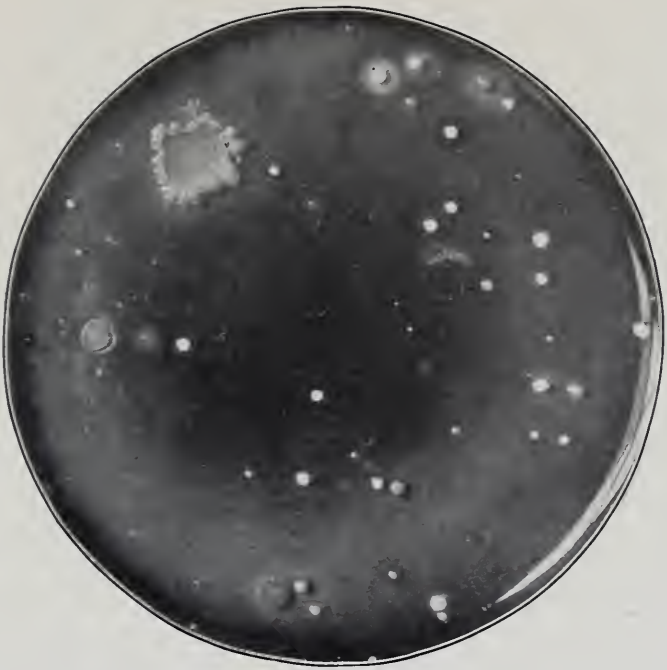
The microscope and stains alone are not enough for complete identification of most micro-organisms. We must use cultural methods as well in order to get pure cultures of the germs and bring out the mass growth characteristics of these pure cultures. In order to obtain pure cultures we must get colony growths from a single individual. We obtain these colony growths by one of two methods. In the first we make high dilutions of the menstrum containing the germ and mix each in a transparent jelly medium while it is in a fluid state; then we pour the mixture into a Petri dish which is made of clear glass, allow it to solidify and let it stand

at the right temperature for growth. In the other method we spread the menstrum thinly over the surface of a solid transparent medium in a Petri dish. Each germ then multiplies many thousand times until there is a little mass growth or colony, usually becoming large enough to be seen with the naked eye. The colonies of many varieties of bacteria present so characteristic an appearance on certain culture media that they are of aid in the identification of the organism. From each of these colonies a pure culture of the organism may be obtained by "fishing" or touching a portion of a single colony with a sterile platinum needle and transferring it to a plate or tube of a fresh sterile medium. In many cases this fishing has to be done under a low-power lens of the microscope, in order to be sure we have touched with our fishing needle no other colonies invisible to the unaided eye (see Frontispiece). The hand of the "fisher" must be steady and his eye true to be able to touch the right colony and only that one.

When we obtain a pure culture we study mass growth further by transferring the growth to special culture media that are suitable for showing up different traits. In this way we examine types of colonies, rates of growth, including counting of colonies, effects on carbohydrates and other substances, and many other traits.

We see, therefore, that in order to begin the study of germs we must know first how to use the microscope; second, how to use stains; and third, how to make and use culture media.

As to the use of the microscope, one can only give here a practical hint or two in regard to focusing.



Different types of colonies of microbes from subway air grown on transparent agar jelly for 24 hours at 36° C. Natural size

Worker ready to fish from colonies of microbes without the aid of the microscope

The chief difficulty in focusing is with the higher magnifications. The objective should be focused down by means of the coarse adjustment until it approaches very near to the glass slide containing the object to be examined, without touching it. This can be observed from the slide. Then with the eye at the eyepiece the coarse adjustment should be moved up slowly and carefully until the specimen comes plainly into view. This focal point may be easily passed if the light is too intense and the specimen thin and transparent. When the object is brought fairly well into focus by means of the coarse adjustment, the fine adjustment should be used to focus on the particular spot desired. When the oil immersion lens is used the objective is focused down until, as observed from the side, it just touches the drop of oil that has been placed on the specimen to be examined.

In examining a hanging drop, the diaphragm of the substage should be nearly closed and the edge of the drop should be found first with a low-power lens.

The influence of stains on our knowledge of micro-organisms has been a most important one. The value of aniline dyes was discovered quite early, but it was not applied to microbes until about 1871, when Weigert and others began staining bacteria, and a few years later (1877) a whole group of investigators began making some important discoveries in regard to the relationship of stains to micro-organisms, chief among them being Weigert, Ehrlich, Gram and Koch. They found that certain bacteria took different stains with different degrees of intensity, and that some were decolorized by certain chemicals and others were not.

So a number of different staining methods were devised that have been of the greatest aid in detecting and learning about germs.[2]

Practically for the identification of most known microbes one needs to know only a few staining methods.

The one that is used most frequently in differentiating groups of microbes, especially of bacteria, is that devised by Gram, hence called the Gram method. The fixed organisms, in spreads or sections, are first immersed for a minute in a strong solution of one of the more intense stains, such as gentian violet which has been made even more effective by the addition of a substance called a mordant, like aniline water. Then the preparation is placed for thirty seconds in some decolorizing solution, like alcohol. About half of the microbes have all of this intense stain removed by the alcohol, while most of the rest retain the stain and appear under the microscope as dark blue or violet germs. The remaining microbes show varying degrees of decolorization. To make those that are decolorized stand out more plainly we finally immerse the preparation in another stain of contrasting color like the red fuchsin in weak dilution. Those that retain the first stain after treatment with alcohol are called Gram-positive organisms, while those that lose it are called Gram-negative organisms. Those that only partly retain the first stain are called Gram-amphophile germs.

The single stain that is most used in practice is one called alkaline methylene blue. When alkali is added to

[2] Such work as Lee's "Vade Mecum" should be read by those who want to learn more about this subject. Published by P. Blakiston's Sons, Philadelphia, 1928—9th ed.

a solution of methylene blue and the mixture allowed to stand for some time, part of the stain is converted into methylene azur, which gives certain granules in some microbes a reddish appearance, while the rest of the organism stains a blue. Diphtheria bacilli are easily spotted by this stain.

Then, certain bacteria, like the tubercle bacillus, and also the spores of bacilli, are so difficult to stain that a strong solution boiling hot must be used; but once stained, they retain the stain even after they are immersed in acid solutions. Such bacteria are called acid-fast organisms.

Special staining methods must be used to demonstrate the capsules that envelop certain organisms, and very special methods must be employed to show those extremely delicate hairlike appendages, called flagella, that motile organisms possess.

The use of culture media in our study of microbes is of course indispensable.

It was early observed that most microbes grew well in various liquids, such as milk, meat infusion, sputum, blood, grape juice, grain infusions, and that they also grow well on solids, such as cheese, sliced potatoes, bread, jellies, fruits, eggs and others; but it was not until methods were devised by Pasteur, Hess, Koch and others for obtaining pure cultures of individual varieties of germs, that knowledge of the kind of culture media best suited for the different varieties of microbes was obtained.

Before we can make culture media we must know about getting everything we work with free from germs —that is, everything must be sterilized. Not only that, but we must be sure that all of the glassware we work

with is reasonably free from acids and alkalis. In order to rid them of these foreign disturbers, each piece must be neutralized and washed in a special way. And the plugging! This means an enormous amount of work in a large laboratory. All narrow-mouthed containers

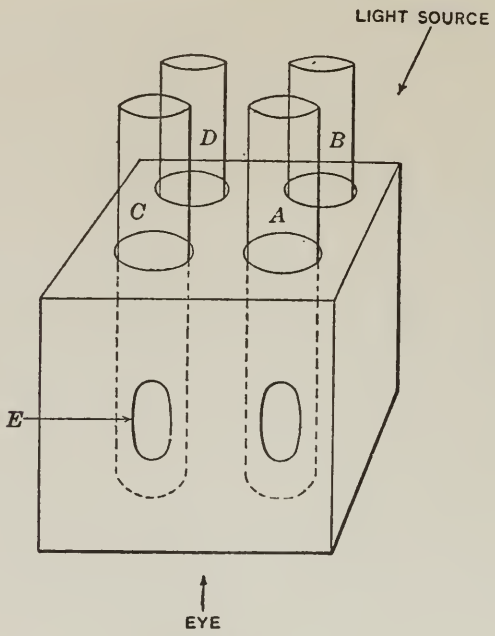



*Fig. 2*

COMPARATOR BLOCK: A, MEDIUM + INDICATOR; B, WATER; C, MEDIUM + NO INDICATOR; D, STANDARD SOLUTION + INDICATOR; E, SLIT FOR OBSERVATION.

used—and there are thousands of them in the larger laboratories—must be plugged with non-absorbent cotton before they are sterilized. This is to protect the interior from contamination, since microbes cannot pass through dry cotton.

A big force of laboratory helpers must be employed

in doing only the work of getting the apparatus ready for culture media. The glassware is all sterilized in dry

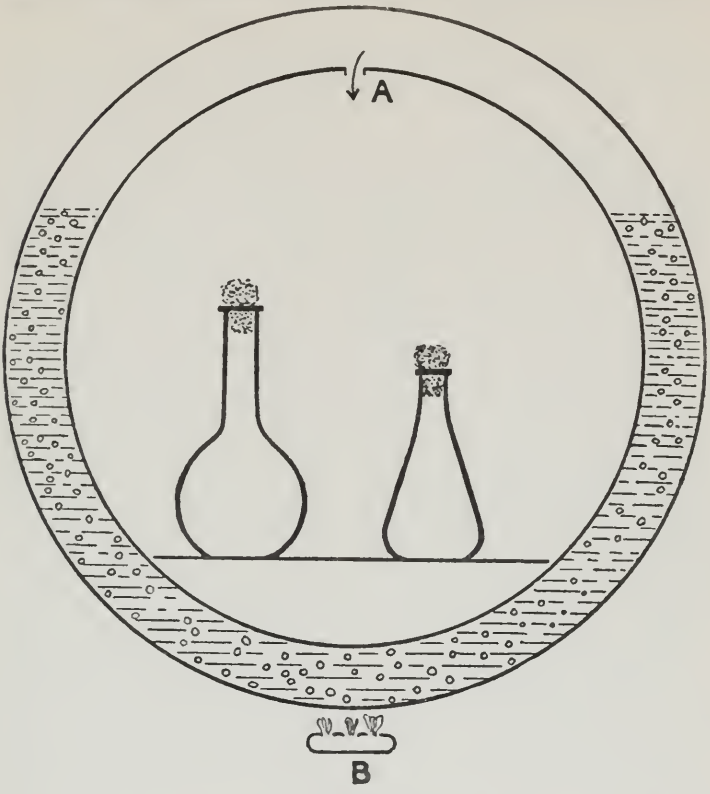



*Fig. 3*

DIAGRAM OF INNER CONSTRUCTION OF HORIZONTAL AUTOCLAVE. A, PASSAGE FOR STEAM FROM JACKET TO CHAMBER; B, SOURCE OF HEAT IF GAS IS USED.

heat ovens at a very high temperature (175° C. for two hours), so that the most resistant spores that sometimes occur may be destroyed. (See plate opposite p. 27.)

It was found that the best culture media for the

largest number of species was a clear meat infusion broth made very much as our cooks make meat broth for our dinner-tables.

We know that beef hearts (what an opportunity for sentimentalists to protest!) make one of the best infusions for our microbes. To this infusion we add a little salt and a small amount of a specially prepared peptone, which is a partially digested protein.

*Fig 4*

ARNOLD STEAM STERILIZER.

The reaction (alkaline or acid) of this broth is a very important matter. While most microbes grow well in a culture medium that is neither acid nor alkaline—that is, one that is neutral—some grow best when the medium is a little alkaline, some when it is a little acid. So each lot of broth is very carefully adjusted for the kind of microbe that is to be grown in it.

We determine these reactions by a special electric apparatus or by a set of colored fluids in test tubes held in what is called a comparator block (colorimetric method). (See p. 24.)

This broth is strained to make it clear and then it is poured into sterile containers and sterilized at a definite

heat, according to the composition of the culture medium and the use to which it is to be put.

The sterilization of media is made in one of two kinds of sterilizers. As a routine we use an autoclave. This is a very strongly built sterilizer, where the steam heat can be increased by means of pressure. We usually use fifteen pounds pressure for half an hour. Some bacteria grow best in a culture medium that has not been subjected to such great heat. For these we use the Arnold sterilizer. At the low heat of the Arnold (about 99° C.), spores are not readily destroyed, so we must expose the culture medium to this heat for half an hour on three successive days, leaving it at room temperature between times. This gives a chance for any spores that may be present to develop into vegetative or young non-sporulating forms, when they are easily destroyed by that heat. When the broth is ready for use it is called nutrient broth. It is not only a good medium in itself for most bacteria, but it is the basis of most of the special culture media that are used for growing the more exacting microbes or growing them for special purposes.

One of the most important additions we make to this nutrient broth is something which will produce a solid transparent medium that allows germs to grow in separate colonies.

It was first found that gelatine was a helpful substance to use. It is fluid when warmed and solid when cooled, so germs can be mixed with it in a fluid condition; and when it was allowed to solidify, all the germs that would grow at the temperature of solidification would grow in little colonies, each one separated from the other. But since gelatine melts at the tempera-

ture best suited for the growth of most pathogenic germs, this was only a partial help. It does allow, however, certain germs to grow in it in a very characteristic way, and so its use was considered an important differential method until other more significant methods were learned. Its liquefaction by certain germs is still used, however, as an aid in classification.

The unsatisfactory characteristics of the animal gelatine were replaced by the very adequate agar agar, a gelatine made from species of algæ in Japan. This forms a very stiff jelly that does not melt under a temperature of 80° C. or more, but when melted does not become solid until it drops to about 36° C.

To these solid nutrient media and to the nutrient broth many different substances may be added to enrich, to differentiate the species and to aid in diagnostic measures; for different germs utilize different substances or change them in different ways.

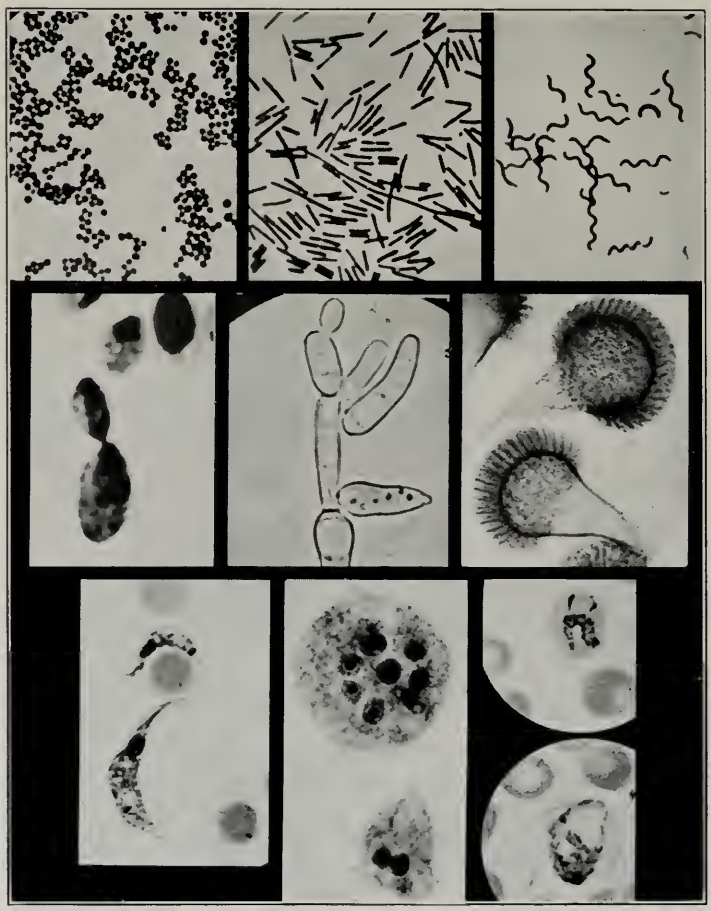
Studying these minute beings called microbes through the microscope, living or non-living, unstained or stained, singly or in masses, in their natural environment or in artificial cultures, we find that they can be divided into three large classes, the bacteria or lowest plants, the yeasts and molds or next higher plants, and the protozoa or lowest animal forms.

These three have been grouped together by some biologists under the name Protista, which means the very first.

Often the term bacteria is loosely used to designate the whole group. The name parasite, which is also applied to the whole group, is not a good general name because many of the forms are not parasitic. Either of

Worker using fermentation tube containing sugar broth for growing microbes, to show their powers to produce acid and gas and grow without air

Corner of media room showing workers preparing glassware to be sterilized in hot air sterilizer, at right. The large flasks containing nutrient broth have just been taken from autoclave, at right foreground



Some types of the three great classes of microbes. Top row shows bacteria—cocci, bacilli and spirilla. Middle row shows yeasts and molds. Bottom row shows protozoa—flagellates, amebas and blood sporozoa. Magnified about 1000 diameters. (From Kolle and Wassermann, except 4, 6 and 8; 6 is from Fraenkel & Pfeiffer.)

the terms microbe and germ is better as a common general name.

Each of these groups has certain general qualities or characteristics which distinguish it from the others, though its border numbers may show some relations to those of the other groups.

First there are the bacteria. The bacteria are considered the simplest of all known living beings. The individual bacterium has no partitions—that is, it is a single cell surrounded by a delicate membrane, like a sausage in a sack. It contains no formed nucleus, as do the cells of most higher plants and animals.

Each bacterium usually contains from one to many minute granules, some of which are composed of nuclein, an albuminous material that is found in nuclei of higher forms. Some of these granules are so definitely arranged and stain so distinctively that, if the bacterium has been fixed and stained in the right way, one may know to which family it belongs the minute one sees it under the microscope. We can spot some potentially pathogenic forms in this way, and so make use of this method to detect them in our diagnostic laboratories. These granules vary in size and coloring according to the age of the bacterium.

At a definite age in the life of a few types of bacteria, mostly found in soils, some of these granules fuse, producing a rounded glistening body surrounded by a thick tough membrane which makes it very resistant to outside influences. These resistant bodies are the so-called spores, seeds or reproductive bodies, about whose discovery we have spoken in our sketch of early discoveries (Chapter I) and whose interference with

the sterility of our culture media we have just mentioned. We have also called attention to the difficulty of staining them and consequently of the very special staining methods we must use to demonstrate them in this way. They are resistant in a varying degree to heat, drying, light and chemical disinfectants. They may lie quiescent (hybernating) for ages, and then when placed in favorable culture media they swell, burst their tough membrane and grow out again to an adult bacterium—the so-called vegetative form. The position of these spores helps in the identification of some forms. Thus, some develop at the end of the bacillus, forming a head like a drumstick; in other species the spores form in the middle of the rod, often making a spindle shape; in others it forms between the center and the end of the rod.

The formations of these spores are said to be nature's simple method of protecting this kind of bacteria against adverse conditions. It is certainly an effective method against many harmful conditions that the germs may meet in the soil which is their natural home. A comparatively few pathogenic microbes are among these marked spore-bearers. Some bacteria that have none of these resistant kinds of spores still have the power to exist in a quiescent state, under certain conditions, for a variable length of time.

The motile bacteria have very fine hair-like appendages, called flagella, which help the little creatures to swim about. When viewed under the microscope some of the motile ones seem to be moving very quickly—and, taking their size into consideration, they do. If man were to move as fast in proportion to his size he would go a mile a minute. Measured by our time they

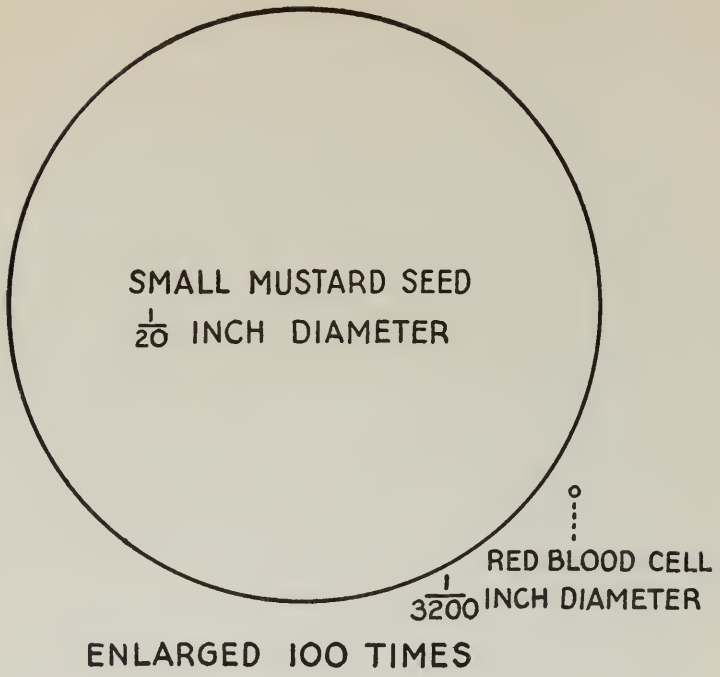
move slowly. It has been estimated that the cholera spirillum at the height of its activity may travel at the rate of eighteen centimeters (seven inches) in an hour.

It is almost impossible to conceive of the size of one of these minute living organisms. To give some idea, we may use some such series of comparisons as follows:

The ordinary high-power lens that we use magnifies about 1600 diameters. A pea one quarter of an inch in diameter if magnified to this extent would look as large as a house about a thirty-three-foot cube. The pea has a diameter about 900 times the length of a tubercle bacillus. If an influenza bacillus, one of the smallest we can see, could be magnified so that it would appear two inches long, an ordinary sized pea magnified in proportion would have a diameter of almost three times the height of the Washington Monument, or in other words it would be as wide as the length of seven city blocks.

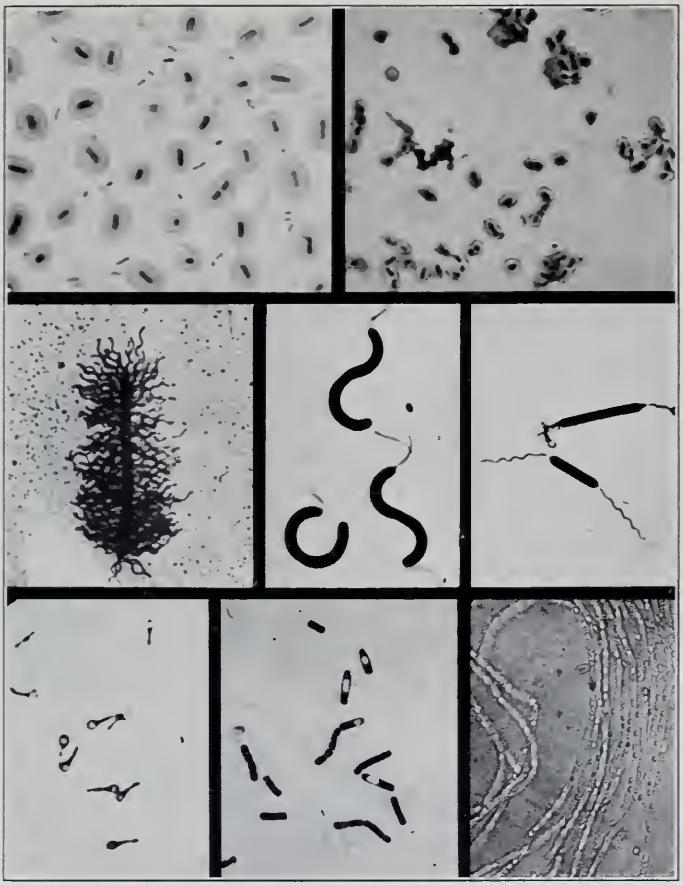
Let us continue the comparison. The unit of bacterial measure is one micron or $\frac{1}{25,000}$ of an inch. The Greek letter μ is used to designate the term micron. An average sized influenza bacillus which is one μ long is one eighth of the diameter of a red blood cell, which is eight μ, or about $\frac{1}{3,200}$ of an inch. A small mustard seed is about one twentieth of an inch. Under a lens magnifying 100 diameters one can just make out the red blood cell and a section of the circumference of the mustard seed (Fig. 5). According to Peabody and Hunt, 1,500 typhoid bacilli arranged in procession end to end would scarcely stretch across a pin head. Kendall estimated that the weight of ten millions of millions would scarcely equal an ounce.

The growth and division of these minute creatures

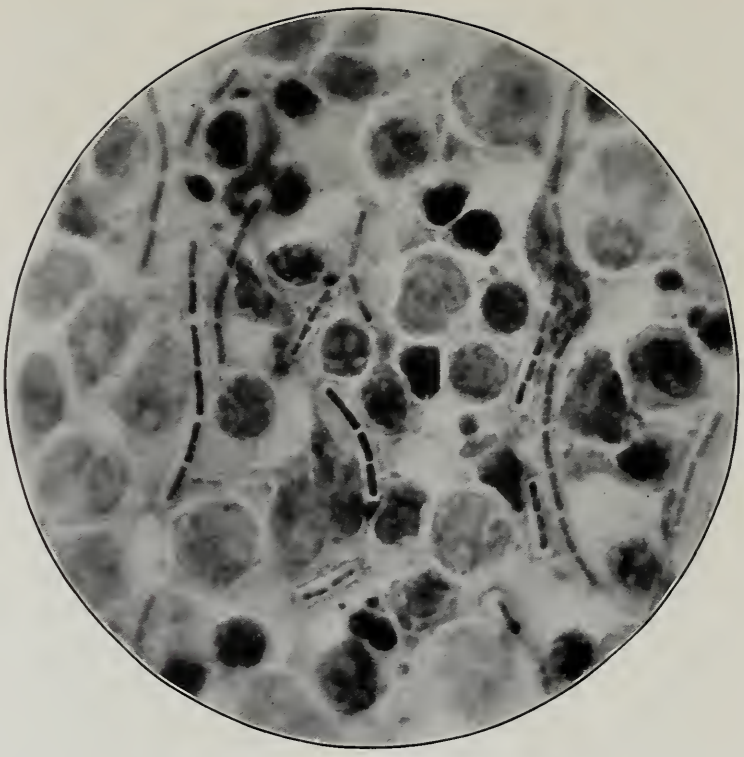



COMPARATIVE SIZE OF BACTERIA

*Fig. 5*



Certain parts of bacteria. Top row shows capsules of bacilli and cocci. Middle row shows flagella of bacilli and spirilla. Bottom row shows spores, in the first growing at the end of the bacilli, in the second near the middle of the rod and in the last the living spores show in threads of bacilli



Anthrax bacilli growing in spleen of mouse and showing a capsule.
Enlarged 1500 diameters

may be observed, if one has patience, under the microscope. We have watched for hours the growth of the diphtheria bacillus and seen the rather startling snapping of the dividing bacilli, usually not in the center as others claim, but nearer one end. The majority of germs, however, divide at or near their middle.

The rapidity of growth varies with the kind of microbe and with the encouragements or hindrances it may encounter as it grows. As the average microbe is said to need only fifteen minutes to divide into two daughter microbes, we can see that with unrestrained growth microbes even in so short a time as twenty-four hours might multiply to an astounding number. But fortunately for us, this continued vigorous growth seldom occurs. Checks to growth are met with on every side: food supply gives out, waste products accumulate, other microbes interfere, the body cells of animals they may attack react and produce substances that may kill them, and other factors all aid to check this mad rush of microbes to increase the numbers of their progeny.

Grouping the bacteria according to their shape, we find they are divided into minute balls or berry-like forms called cocci, minute rods, or elongated sacs, like sausages, called bacilli, minute parts of spiral rods called vibrios. Then there are more definite longer spirals called spirilla and spirochetes. Finally, there are thread-like forms with branches called higher bacteria because they resemble the molds. These different characteristics help to divide them into families which will be described later.

There is no one distinctive characteristic that separates these low plant forms from the simplest animal

forms, but when a form shows somewhat more completely its ability to grow into filaments and branches, with definite partitions as in the molds, it is placed in the next higher plant class; while if it shows a more definite nuclear apparatus with a more plastic body and with more complex reproductive powers it is placed with the protozoa.

The molds, including the yeasts, are very common in nature, and the characteristic ones are easy to recognize and place. But there are many varieties of this big group that have not been minutely studied, so there is still great difficulty in classifying them. A number of them have sexual phases in their growth. They all form spores that are quite resistant to drying, and as the spores of many varieties germinate when only small amounts of moisture and food material are present, a moldy condition often prevails.

It is interesting that while not many pathogenic yeasts or molds are known, one of the first diseases in which a micro-organism was found was one caused by a mold. Schoelein found it in 1839 in a disease of the scalp called favus; in which little yellow cups of the mold grow around the base of the hairs. Shortly after this Langenhock discovered the thrush parasite, which is a yeast-like mold that so often grows in white patches in babies' mouths.

The yeasts, or budding microbes, usually have a very refractile membrane which is easily identified under the microscope. While most yeasts are larger than most bacteria, a filterable form has recently been reported by Lewis. This is evidence, of course, that very minute forms of this kind of microbe exist.

The majority of yeasts and molds grow easily on a

food medium containing a little sugar and that is not too alkaline, but some of the pathogenic forms, especially of yeasts, seem very unwilling to start growing on artificial media. Once started, they too grow readily. On the whole, molds prefer a slightly acid food medium.

The yeasts in general stain readily, and are irregularly Gram-positive and Gram-negative (amphophile). The molds usually require special handling before they can be stained. Then they also are Gram-amphophile.

The protozoa, because of their larger size and more differential structure, are usually more easily studied under the microscope, but they are more difficult to grow in pure culture. Few accommodate themselves to the ordinary laboratory culture media. Quite special media must be used to obtain satisfactory growths. These will be described later.

Though the protozoa are larger and show more structure, most of them pass through such a complex life cycle, often part of it in a different kind of animal from the original host, that unless the whole cycle is known individual forms may be very difficult to classify. Thus a flagellate form found in a mosquito may be a stage in the life history of the ameboid malarial parasite found in the blood of human beings. Their study is further complicated by their having sexual and asexual phases.

While most protozoa are much larger than most bacteria, there have been forms described that are small enough and plastic enough to pass through some of our finer filters.

## CHAPTER III

## HOW MICROBES LIVE AND ACT

How they behave under different conditions—Helpful, harmless, harmful activities—How they are carried to individuals and from one individual to another.

Microbes are almost ubiquitous. Members of each class may be found all over the known world. As long as a trace of organic substance of the simplest nature and a little moisture are present, germs of some sorts may grow. They must all find at least the elements, nitrogen, carbon, hydrogen, oxygen, phosphorus and sulphur, but some can make use of them even in their elementary form and others require all grades of combinations. Most varieties need very complex food.

There are kinds that can grow on snow, and kinds that grow in hot springs; but only a few varieties can grow at such extremes. Some varieties have been found by polar explorers as near the Poles as man lives, while in the tropics many varieties are found in great abundance. They are found in the ocean, in the air and in the upper layers of the soil. Indeed, the upper eight inches of soil constitute perhaps the greatest breeding place for many microbes. They grow easily, multiplying exceedingly under favorable conditions, but then, fortunately for us, most of them die easily under unfavorable conditions.

Little wonder that many varieties of microbes have

been evolved through the ages when they evince such ability to grow under so many different conditions, their power to adapt themselves to their surroundings. There are not only the kinds that grow best with the simplest food, or with the most complex food; those that can live best at high temperatures or low temperatures; but there are those that carry on their lives best under more special conditions. Indeed, some will not grow at all unless certain factors are present or absent.

For example, there are those that grow only in the presence of free oxygen and those that will not grow unless free oxygen is removed; there are those that feed on certain sugars, others that feed on none; those that like to live on or in human beings or other animals, or those that choose plants for their homes. There are even some that are so fastidious in their tastes that they grow only in certain species of animals or plants.

Germs have been given special names to designate their behavior toward their surroundings. These names are often high sounding, though their meaning may be simple enough. Thus, those microbes that seem to depend only upon a living host for their existence are called pure parasites; those that grow only on non-living food are called pure saprophytes if plant germs or pure saprozoa if animal germs. Those that have the faculty of growing either on living or on dead matter are called facultative saprophytes or saprozoa.

Those germs that grow best at low temperatures are called psychrophiles or lovers of the cold. The red and the blue snows, which for a long time seemed so mysterious, are caused by germs of this kind that produce either a red or a blue pigment. Many of the germs that grow in the cold water of the ocean belong

to this group. All forms that grow only below 30° C. are placed in this group. The temperature the majority like best—that is, their optimum temperature—is 18° C.

Those germs that grow best near the temperature of the human body are called mesophiles or lovers of the moderate. The pathogenic germs of human beings belong in this group. Temperatures a little above or below this blood heat may have a marked inhibitory effect on their activities. In order to keep our cultures of poisonous germs at this equable heat we use special incubators.

Those that grow at higher temperatures are called thermophiles or lovers of the heat. They grow best at about 50° C. They are found chiefly in the soil and in the alimentary canal of some animals. Some are found in certain hot springs with a temperature of 140° to 170° F. Ordinary plant or animal life could not live at this temperature. Some of these thermophiles make great difficulties in the pasteurization of milk where long runs are made. They increase as the others decrease.

While germs cease all movement and growth at unfavorable temperatures, they are not so easily killed by cold as they are by heat. Only a comparatively few degrees of heat will kill most forms in a short time, while many degrees of cold applied for a long time are required to kill the majority of the varieties. Thus fresh ice may contain a large number of germs, while older ice may have few or none. Alternate freezing and thawing is more detrimental than continued freezing. The age of the germs and the media in which they are growing influence to some degree the effects of heat and

cold. Older microbes immersed in an albuminous or an oily substance are much more resistant to heat or cold, either continuous or alternate, than are those not so surrounded.

The fact that so few microbes of any kind, especially disease-producing microbes, live at the extremes of temperature has led to the use of methods for the preservation of foods in which the application of either heat or cold is used. That useful method of keeping down microbal growth, called pasteurization in honor of Pasteur, developed from our knowledge of the deadly effect of heat on germs. This use of moderate heating has developed into a great industry in the pasteurization of milk.

Those that require the air or free oxygen are called aërobes; those that do not, anaërobic. Those that can live either with or without free oxygen are called facultative aërobes or anaërobes. We have special methods of growing our anaërobic microbes.

Microbes pathogenic for man as well as some saprogenic forms do not like certain of the sun's direct rays. The rays at the ultra violet

*Fig. 6*

BUCHNER'S ANAËROBIC TUBE. THE FLUID CONSISTS OF PYROGALLIC ACID DISSOLVED IN 10 PER CENT. NaOH SOLUTION. BY WILSON'S METHOD THE TUBE IS CHARGED WITH PIECES OF CAUSTIC POTASH COVERED WITH PYROGALLIC ACID.

end of the spectrum have a killing power over most of the pathogenic forms. Window-glass will absorb these rays; quartz permits them to pass.

The tubercle bacillus particularly has been under observation as regards the sun's rays. It has been found that in the presence of abundant oxygen and sun's rays the germs in sputum may be killed in a few hours.

The inhibitory effect of direct sunlight on certain bacteria may be easily demonstrated by mixing the bacteria into a jelly medium when fluid, such as warm nutrient agar, and pouring the mixture into a series of quartz Petri dishes, then covering a part of the cover with a strip of black paper. These are then exposed to the sun's rays for a variable time and put at the proper temperature for growth. After a certain exposure colonies of the germs will appear only over the protected areas.

Radium rays and electric rays also have a killing effect on microbes directly exposed to them.

The study of the effect of electric charge or the electric potential difference between a germ and its menstrum may lead to new methods of recognizing specific varieties. Falk [1] has summed up the recent studies on this suggestive subject and outlined a theory of microbic virulence.

The influence of direct pressure upon microbes has been found to be slight so far as their destruction is concerned, but the influence of differences of pressure between the culture medium and the germs—the so-called osmotic pressure—is of great importance upon the growth of microbes. Upon osmosis depends largely

[1] "The Newer Knowledge of Bacteriology and Immunology." Ed. by Jordan and Falk. University of Chicago Press, 1928.

the growth of all germs. The difference in pressure between the cell and a new medium must not be too great or the cell may either swell and burst or become shrunken and dried in its efforts to establish an equilibrium.

Thus, if a strong salt solution is added to a collection of microbes, the water in the germ cells will be attracted to the salt and will pass out of the porous membrane enclosing the cell and mix with the salt solution. Depending upon the amount of salt and water, a certain amount of salt may pass into the bacterial cell until the pressure is equalized. In the process the germ ceases to grow, and it may actually die. A practical application of this principle is known as pickling or corning.

Strong sugar solutions have the same effect and are also used to preserve foods from the growth and deleterious effects of microbes. The Frenchman Appert used this principle long ago in the preserving of fruits.

The inhibiting effect of drying upon microbes was used in practice long before it was known that microbes existed. Meat and fish were and still are preserved by the process of "jerking." This is simply the cutting of the fresh meat and the hanging of it in the sun to dry after cutting the larger portion into thin strips. Now desiccation is practised in the industries on a large scale. Desiccated fruits and vegetables, desiccated meats and fish, desiccated milk are all well preserved and on the market.

Microbes growing together may have a marked effect on each other for good or for evil. Certain products of the growth of one may help directly the growth of another, thus acting as a vitamin. For example, the influ-

enza bacillus will not grow alone unless blood or certain vitamin-like substances from different vegetables are mixed with ordinary culture media, but it will grow on ordinary media in mixed cultures with certain microbes. This is called symbiosis, though it is not symbiosis according to the strict meaning of the word. Some microbes, however, grow in true symbiosis with certain plants. On the other hand, certain germs are antagonistic to each other.

Certain germs, even harmful under some conditions, may grow on the surface of man's body without doing it any apparent harm. These are the so-called parasitic forms. While these germs may not be harmful to the human being carrying them because he may not be susceptible to them, they may be transmitted to other people coming in contact with the secretions or excretions containing these germs—*e.g.*, saliva, through kissing; and if people are in a susceptible condition they may come down with the disease.

People apparently normal who harbor germs of potential pathogenic power are called human microbe carriers or human vectors. It is in this way, by the human carrier, that certain diseases continue to be spread. Fortunately for us, pathogenic germs, as a whole—that is, those that are highly parasitic—do not exist for a long time outside of the animal body, except under very favorable conditions. When expelled and exposed to the natural germicidal agencies of sunlight and desiccation only a few may reach a new host. This is our salvation. Spore-bearing forms and any facultative parasites are exceptions to this rule. The closer the relationship in time and space to the source of infection, the greater the chance of successful transfer

of virulent infective agents. Germs have become adapted to escape by the natural avenues used for the physiologic discharge of secretions or excretions of the body —that is, the sputum, feces and urine. The easier the avenues of escape from the host, the greater the number of germs that infect a new host. Those infectious germs that can readily reach and infect a new host are called contagious. All germs that are poisonous for man are called infectious; only a few of these are labeled contagious.

The carrier may have contracted the carrier state after a regular attack of the disease, when he is called a convalescent carrier; or if he has some immunity when he takes in the germ after contact with one who is harboring it, he may become a carrier without contracting the disease. In this case he is called a normal or a contact carrier.

Carriers are called transient or temporary if they have the germ for only three months or less. If they harbor the germ longer, they are called chronic carriers. The carrier may be more dangerous as a power of infection than an actual case, because he may live unsuspected in a community.

Some germs spend part of their lives in each of two hosts. Bacteria for the most part are only adventitiously carried by lower animals. Thus the house-fly may carry typhoid bacilli on its legs after walking over infected feces or other infected material. Protozoa, however, have the faculty of carrying on part of their life cycle in a lower animal, usually an insect carrier or vector, such as the mosquito in malaria. Such a lower animal may be the real or definitive host of the germ, and the higher animal the intermediate carrier. Some

pathogens for man have a higher animal as a natural host—for example, rabies in dogs.

Germs in their growth not only manufacture products that have helpful or harmful effects on each other, but they form products some of which, as we have said, are helpful, even essential, and some harmful to man. The power of microbes to do this depends upon many factors, to some of which we have alluded.

To tell all we know in a book of this size about the helpful and harmful products of microbal growth will be impossible. We can only touch here and in later chapters on the more important ones that occur in the industries and in disease. The influence of many of these factors may be readily shown in the laboratory. Thus, the diphtheria bacillus has the power to produce a very potent toxin which can kill human beings quickly if it meets with no obstacles.

The discovery that this toxin could be produced in the laboratories was made many years ago by two Frenchmen—Roux and Yersin. That was a wonderful discovery. Their original method of manufacturing this toxin, due to their knowledge of only a few of the factors influencing its production, was long and complicated. They used large flat flasks, containing only a little of the culture medium, each flask connected in series to cylinders of oxygen gas. The gas was drawn through the flasks by a suction pump. It was thought that oxygen helped the growth of the bacilli and their production of toxin. The bacilli were kept in this condition for about six weeks.

This was the lengthy and cumbersome method that was still being employed when we started our investigations on the best method of producing this toxin. We

quickly made some discoveries in the New York City Health Department laboratories that altered our ideas as to how best to make this toxin.

In the first place, we were fortunate enough early to find a strain of the diphtheria bacillus, among the many we tested, that produced, under the right conditions, a very strong toxin. We found, further, that neither this strain nor any other we tested needed the stream of oxygen to make it produce good toxin; moreover, we found that the toxin appeared in largest amounts in about six days instead of six weeks.

We found also that the reaction of the culture medium was of the greatest importance. If the medium was a little too acid or too alkaline, no toxin or very little would be produced. So on the addition of a certain amount of sugar to the medium, the bacilli in their growth may produce no toxin.

It is a commentary on our lack of knowledge of these mysterious creatures that, notwithstanding our taking advantage of these and other new facts learned by investigators throughout the world, we still get variable results in trying to produce good toxins for the manufacture of antitoxins and for the vaccines used to protect children against diphtheria. We have found recently that these bacteria as well as all others we have studied tend to change and produce non-toxic varieties, and we are learning how to keep the toxic varieties in full vigor.

The kind of a toxin produced by the diphtheria bacilli is called an exotoxin, because it is found in the culture medium outside of the bodies of the bacteria themselves as a result of their growth. Toxins of this nature have the power, under appropriate conditions,

of stimulating in the susceptible animals injected with them substances that counteract the poisonous effects of the toxin, the so-called antitoxins, which will be described in the next chapter.

The outstanding toxins of this type are only four, diphtheria, tetanus, botulinus and scarlet fever. Their traits will be taken up in later chapters.

There are a number of other extracellular products of a similar character that have various effects upon the medium in which they grow. They are called enzymes or ferments, and they produce reactions such as coagulation, liquefaction, digestion, decay, putrefaction, sugar and fat splitting, and so on.

Many of these reactions are essential in the cycle of life. Some of them are taken advantage of in identifying closely related microbes and are used to detect epidemic strains.

The most striking characteristic of microbes is their individuality combined with their variability. If we were able to learn all the traits of each individual microbe and how these traits varied under different conditions, we would no doubt find an individuality and a variability comparable to those we observe among more complex living forms. The progeny of each microbe exhibits, for a time at least and under similar conditions, the traits of its ancestors. This individuality is called specificity. Many species of microbes have such specific individual traits that they may be recognized more or less easily by their characteristic growths and reactions in different culture media, including their growth in the body of a living host.

One of the most important types of substances used by microbes as well as by man in their growth is that

known as carbohydrates. Carbohydrates are the chief source of energy of life. They comprise the sugars, starches and related forms. There are hundreds of them, some well known to us, such as cane-sugar, grape-sugar, milk-sugar; others known to chemists and microbiologists as important chemical reagents. Certain microbes ferment certain of the sugars, others few, others one and others none.

In using the sugars for testing the relationship of microbes one must be very sure of their purity. We make the final test by using known microbes that may detect the smallest quantity of a particular sugar, so we always have a number of control tubes implanted with different test microbes whenever we make a sugar test. Microbes are among the most delicate and specific testers of sugars.

Some microbes split up the sugar with the production of acid and gas, others only with the product of acid. We use a special bent tube with one end closed when we wish to show the presence of gas.

The presence of acid is demonstrated by putting into the test medium a substance that will change color if the germs split up the sugar with the formation of acid. Such a substance is called an indicator. The chief ones used by microbiologists are litmus, phenolphthalein and certain dyes decolorized by sodium hydroxide, or reduced by sodium sulphite.

A rather complex series of media of this type that is very satisfactory is used in demonstrating the presence of different members of the intestinal bacteria, the so-called typhoid-coli-dysentery group.

There are a number of other products of microbal growth classed among the enzymes that have a marked

effect upon different cells. Some cause a breaking up or dissolving of red blood cells. These are called hemolysins. Others affect other cells. They may all be classed under the general name cytolysins, or cell dissolvers.

Besides producing these exoenzymes and exotoxins, some micro-organisms have the power of injuring the tissues of their hosts by growing directly in and through them. The germs either release a poison as they die (endotoxin), or they use certain parts of their hosts' cells for their food and thus destroy the cells directly; or they form a poison out of this new combination of bacterial activity and cell breaking up. All of these poisonous effects are said to be due to endotoxins.

Whatever the nature of this poisoning, the chief practical point is that, when susceptible animals are injected with non-lethal graduated doses of such micro-organisms, specific antibodies or counter-poisons appear in the serum of the injected animals.

These antibodies are quite different from the antitoxins stimulated by the exotoxins. They are more complex. They will be described in the next chapter.

Among these substances is one that still remains a great mystery, though many studies have been made in attempts to discover its true nature. This is the famous "bacteriophage" of Twort and d'Hérelle. We don't yet know whether it should be described here as a toxin-like ferment, or be put with the filterable viruses as a living entity. Some writers declare that it is one, some that it is the other.

Here is the story of its discovery, and the little evidence we have in favor of each view.

In 1915 an Englishman of the name of Twort, while searching for the cause of vaccinia (cowpox), noticed,

in some of the dense colonies of bacteria growing on the nutrient agar culture medium he was using, some translucent or watery looking areas. The colonies in which these areas had advanced farthest could not be subcultured, and if left long enough the whole colony would become translucent. If a small portion of such a glassy colony was touched to a pure culture of the same type of bacterium as that in which they had appeared, a spreading, translucent area started, and soon the whole culture might be changed and become incapable of being transferred; that is, it was dead.

This power to change the growing culture to a dead glassy mass, was possessed by filtrates of this translucent material. The active principle passed through the finest porcelain filters without any apparent loss of activity.

Twort thought that this active agent might be a living filterable virus, but he favored the hypothesis that it was an enzyme derived from the bacteria themselves.

No attention was paid to this work until 1917, when the Frenchman d'Hérelle announced as a new discovery that he had found a living, ultra-microscopic organism that killed certain bacteria. While d'Hérelle was attempting to discover the cause of the disappearance of the dysentery bacilli from the feces of convalescents, he found that minute quantities of fecal filtrates of such patients inhibited the growth of dysentery bacilli in cultures. He found, just as Twort had, that this lytic power could be passed on from culture to culture indefinitely—indeed, that it seemed to become more active the longer it was transferred.

D'Hérelle believed then, and he still strongly be-

lieves, that this phenomenon is due to a living, multiplying ultra-microscopic microbe that destroys certain bacteria. He has even given it a name. He calls it Bacteriophagum intestinale.

Which idea is right? In their search for an answer to this question many new facts have been discovered in regard to this phenomenon, but the question has not been settled yet.

It has been discovered that many bacteria are subject to this phenomenon. Almost every bacterium studied from infected material seems to have an associated phage. While the evidence is in favor of each phage being specific for each micro-organism or group of micro-organisms, this too is not proved.

Each evidence that is brought forward in favor of a phage being a living principle is shown by the opponents not to have been sufficiently controlled. One should read Bronfenbrenner [2] to get a summing up of both sides, and the latest researches on this subject.

The questions of resisting cultures, of the production of antiphage, and of the significance of phage in infectious diseases, are all of the deepest interest.

According to d'Hérelle and others, phage may play a very important part in the cure of certain diseases. Good results have been reported by some in staphylococcus infections such as boils, in dysentery, in cholera and in a few other diseases. Others have not been able fully to corroborate these results.

[2] In "Filterable Viruses." Ed. by Rivers. Baltimore: The Williams & Wilkins Company, 1928.

## CHAPTER IV

## HOW NATURE REACTS TO MICROBES

Limitations of nature's methods—Man's assistance—Natural and acquired immunity to poisonous microbes.

"Let nature take its course"; "Do not interfere with nature's plan," or a similar cry is often heard from some slogan crier among the superficial observers who are all too frequently heard in this easy-going world of ours. Such people do not realize that there is no clear-cut dividing line between man and the rest of nature, that man's attempts to regulate the ways of the microbes is a part of nature's general scheme; in other words, that man himself is one of nature's forces. We want to show, therefore, in this chapter how nature reacts in this broad sense of the meaning of this phrase.

Let us rehearse first what might happen if we should "let nature take its course" according to the way these narrow "naturalists" interpret the meaning of the phrase. In other words, how would the world be affected if we humans stopped guiding and controlling all microbes and tried to continue our communal way of living?

Perhaps the first thing to be affected throughout the civilized world, at least during the warm weather, would be the transportation of milk. For milk, our most important single food, the food for babes, for the sick, for the old and young alike, is also a very good food for

germs. So if man does not cool it soon after it is taken from the cow, to keep down the number of germs present—and there are always some, however carefully the milk is collected—the germs would grow rapidly and the milk would soon be spoiled or at least become disagreeable as food for humans.

Moreover, if the milk had not been extraordinarily guarded in its collection and in the choice of the cows, or if it had not been pasteurized, disease-producing germs that might be present could increase to dangerous numbers.

We would have to go back to the old practice still in vogue in some parts of the warmer countries, as in sections of Cuba or Spain—that of having a goat taken about from house to house and milked at the doorstep; or, as in earlier days in many parts of the world, that of taking our bottle or pail to the cow's stable so the milk could be obtained and used fresh. Or we might allow milk to become naturally soured, as they do among nomadic tribes or in some tropical countries where they cannot get ice. Of course, they take unconscious advantage of microbal activity in allowing the bacteria to grow and produce the acid that sours the milk quickly and so interferes with the action of the putrefactive bacteria.

All the products associated with milk as a commercial industry—cream, butter, cheese—would be affected, at least in production on a large scale, where microbes are used for starters, flavors and colors. In short, the dairy business would be practically wiped out. If man stopped his control of germs, even the wholesale manufacture of desiccated milk and completely dried foods in general, which are prepared to control microbal growth,

would also cease, though this method was used at first by individuals not knowing of its effect on microbes. Other industries depending upon refrigeration, such as cold-storage plants, and so on, would also be eliminated.

Then industries growing out of the use of high temperatures for killing microbes or stopping their growth would cease. Pasteurization of milk has already been mentioned. The whole canning industry, including the making of condensed milk, would be shut down.

Business depending upon the conscious use of microbes for aiding in certain processes, such as the tanning of leather, the retting of flax, the production of ensilage, the production of alcohol through yeast, the making of bread with yeasts, and many other industries—all of these would disappear as industries on a large scale. Even if the making of wines and beers did continue for a while, their products would become undesirable or, as Pasteur said, "sick," through their being contaminated with microbes that develop disagreeable tastes and smells, or with microbes that interfere with the growth of the yeasts and produce products like lactic acid instead of alcohol.

Food poisoning, with its tragic spectacular effects, would increase.

Fruit and vegetable growing on a scale large enough to meet the needs of great masses of people would gradually cease—first, because of blights due to the lack of spraying, and second, because of soil depletion from intensive cultivation. Of course, barring these two hindrances, the life cycle of plants and animals would go on, but agriculture as an industry would cease.

Animal raising on a large scale likewise would be

greatly interfered with, due to uncontrolled epidemics, the lack of the right kind of fodder and the proper care of waste products.

Last would occur the disappearance of large communities of civilized man through disease and starvation. The human beings that might survive would be forced back to a nomadic life, taking nature as they found her, moving with game and away from soil depletion and spoilage, eating meat even then preserved by natural freezing or drying, eating maggots or ants as titbits. Thus a proportion of survivors might exist for a "natural" span, as did early tribes and as do the nomads of to-day, such as the most northern Eskimos, who move from place to place as their sources of food supply change.

Whatever care nature takes of these tribes is done without their understanding how or why. They only know that they are constantly facing disease and starvation if they do not move to new quarters where game and soil are good.

Those who earlier decided that they wanted to live in larger groups in settled homes found that in order to do so their food and water had to be brought to them freed from harmful microbes, and that their waste excreta had to be taken away by watercourses where dilution, sedimentation and oxidation would lessen their menace; or they had to be otherwise made innocuous by such means as filters or chemicals. And so our towns and cities with their sanitary codes grew.

Even if man should exercise partial control over microbes—that is, if he should ice or sterilize his foods when necessary for their preservation, dispose of his waste products or sewage in an ordinarily hygienic

manner, spray his fruits and vegetables sufficiently, see to it that his soil has the proper nitrogen-fixing bacteria and other essentials for the cycle of life, even then he still would have to contend with the pathogenic germs that live in or on himself and other animals.

How would these act if man did not try to control them and employ them? Does it look, to use the figurative language of our sentimental naturalists, as if "nature wants man or the microbe to survive"? The story of the black death, of the fight against yellow fever and pernicious malaria in the building of the Panama Canal, of the typhoid fever and cholera tolls in India and other parts of the world, of the great influenza pandemics, must point these misguided ones toward the view that the microbe is, at least sometimes, a favorite of Dame Nature, who, if not interfered with by that "unnatural product" man, will assist the microbe in unknown ways to develop and strengthen its toxic powers and other weapons of offense against man.

"But," these people cry, "some people recover spontaneously from disease due to epidemic microbes and other poison-producing germs, even after the most severe attacks, so nature must sometimes assist man too. How does she do this?" "It is true," the thoughtful ones answered the first questioners, "and we may be able to find out why some animals recover from disease and some die, if we do more studying." So they kept on thinking, observing, questioning and experimenting.

It was early noted that people and other animals who recovered from certain diseases seldom or never had another attack, and that when they recovered from certain other diseases they seemed to get another attack

as readily. Why? How? continued to ask the few. The attempts to answer these puzzling whys and hows constitute the history of our knowledge of the controlled prevention and cure of the infectious diseases. Bit by bit these searchers learned something of how nature attempts to protect man against the inroads of the few but very dangerous pathogenic microbes, and how man can improve these methods. We learned that nature's methods are incomplete and haphazard. We learned that man, guiding, assisting and controlling nature in these haphazard methods, is able, with the knowledge gained from his investigations, to devise clean, controlled and intensified improvements of these methods that are many times more effective in the prevention and cure of certain diseases than are the slow uncertain methods of unassisted nature, with her tragic epidemics.

To illustrate one of the ways developed by man for improving nature's methods—in other words, to show how he must be considered as a part of nature in the broader sense—we will give a brief sketch, first, of how nature unaided by man takes care of a child who becomes infected with a toxic diphtheria bacillus, and second, how man has aided to make this care better.

The child gets these germs into its throat, at school or elsewhere, usually from a playmate, who either may be just beginning to have diphtheria or may be only a healthy carrier of the germ. The bacilli may be transmitted either directly through the sputum (spit) by the sputtering of the germ carrier in talking, coughing or sneezing (droplet infection), or by kissing, or by other contact such as infected hands, hands that so often go into the mouth or nose of even the best brought up child; or the bacilli may be carried indirectly

through using a pencil or other article the carrier has had in her mouth, or through tasting a bit of her sandwich or other food, or even having a chew of her gum.

In the "good old days" of nature's methods unaided by man it was not known that there was such a thing as a healthy human carrier of dangerous microbes—that is, a person who is either naturally immune or has developed immunity through having had an attack of the disease, or through vaccination (acquired immunity); nor was it known that germs are conveyed by infected droplets of sputum expelled through the explosive talking of the carrier.

People thought in those days that the air continued to contain these vicious germs indefinitely, and they devised elaborate methods for fumigating the air after a patient had recovered from a contagious disease. A large part of the force of public health departments was made up of fumigators, and a considerable laboratory force was employed to carry out the tests thought necessary to show that the fumigation had been thorough.

But after carriers were discovered and the chief source of new cases of infectious diseases understood, it was learned that such elaborate fumigation methods were usually unnecessary. It is true that the fine spray from the mouth in coughing or explosive talking or from the nose in sneezing may often reach far, but these microbe-containing droplets do not stay suspended in the air. They fall upon any near surfaces and, as they dry, the vast majority of the poisonous ones quickly die. Of course, when careless people crowd together, as in meeting-houses of any kind or in buses,

trains or trolleys, danger through droplet infection is great.

In the fumigation period, not only did they know nothing about healthy carriers, but they knew no way of showing, on the one hand, that one child was susceptible to diphtheria—that is, that it had no power to resist the inroads of the microbes—and, on the other hand, that another child was not susceptible; that is, that even if it took in the diphtheria bacilli, it would not come down with diphtheria.

Now with the aid of a special test we are able to show which children are susceptible to diphtheria and which are not. This test, which is called the Schick test, so named after the one who first demonstrated its use, will be described later.

If the child is very susceptible the bacilli, once getting into its throat, will grow there and produce that very strong toxin (exotoxin) that we described in Chapter III. The toxin will pass through the whole body of the child, causing a very severe attack of diphtheria, and the child may die. If he recovers, it means that his tissues had the power to respond to the attack of the bacillus and its toxin by manufacturing counter-toxins or antibodies that neutralize the effect of the bacillus. If he only made just enough antibodies to combat that attack, he might be left with very little or no power of resistance against another attack. Such children, if left to nature, might have two or more attacks. If man did his part in the workings of nature he would make the Schick test on a child and would be able to judge from that whether that child could be assisted in the production of diphtheria antibody (antitoxin) by giving several doses of diphtheria vaccine. You will

learn later that this has been done successfully on a very large scale, first among the children of New York City and then throughout America and other parts of the world.

If a child has a little power to resist the diphtheria bacillus he may have a lighter attack of diphtheria, because his tissues respond more readily to the attack of the bacillus, producing more antitoxin. After he recovers he usually has a greater immunity, which may last indefinitely, and he may never get another attack of diphtheria. Of course, such children respond very readily to the vaccine and remain immune probably forever after once being vaccinated.

The reaction of the human host in successfully combating infection by the diphtheria bacillus illustrates the way in which one kind of antibody is produced and how it acts. That is, the body cells of the host manufacture an antitoxin that neutralizes the soluble toxin (exotoxin) formed by the bacteria.

Certain microbes, as we have said in the preceding chapter, manufacture another kind of toxin, the so-called endotoxin, which is not given out freely from the microbe cell body in their growth, as is the diphtheria bacillus exotoxin. It is supposed to stay in the cell body until the microbe breaks up in death; or the pathogenic action of certain forms may be the result of toxin formed in the extraction of host cell substance while the organisms are growing there.

At any rate, the antibodies stimulated in the host by this type of poison are more complex than those stimulated by the exotoxins. They cannot neutralize the harmful action of these microbes without the help of another substance that is normally present in the blood.

This substance is called complement because it aids the antibody. When a pathogenic microbe of this type attempts to invade an immune animal, this complement cannot act until the specific immune body, or antibody, attaches itself to the germ; then the complement unites with the immune body—in technical terms, becomes fixed to it and helps dissolve the germ membrane, releasing and making innocuous the cell contents and killing the germ. Neither antibody nor complement can act alone.

This kind of specific antibody may be greatly increased in a person or animal by repeated doses of the specific vaccine. The serum also of animals that have received many doses of the vaccine may have some effect in curing certain infections, but the practical effect is not so marked as is that with antitoxin serums. These serums are called antimicrobal or germicidal or lytic serums. They are called lytic because they dissolve the cells that were used to stimulate their production. Not only microbal cells but other animal and plant cells may stimulate the production of a similar serum. Each serum is specific; that is, it reacts only on the cells that were used to stimulate its production.

This fact is made use of in the employment of a well-known test called the complement fixation test, one form of which is the very famous Wassermann test.

Both of these tests are used chiefly in helping to detect infections caused by special types of micro-organisms.

The Wassermann test is used to detect syphilitic infections. There is no need to add that this test is in frequent demand. A considerable part of the laboratory force in most of our so-called diagnostic labora-

tories is employed in carrying out this test. It is a very complex one and needs expert technicians to carry it out in a satisfactory manner.

Another important diagnostic test is made with serums stimulated by the injection of specific microbes. This is called the agglutination test. Serum from immune animals contains a substance called agglutinin, which, when the serum is added in appropriate dilutions

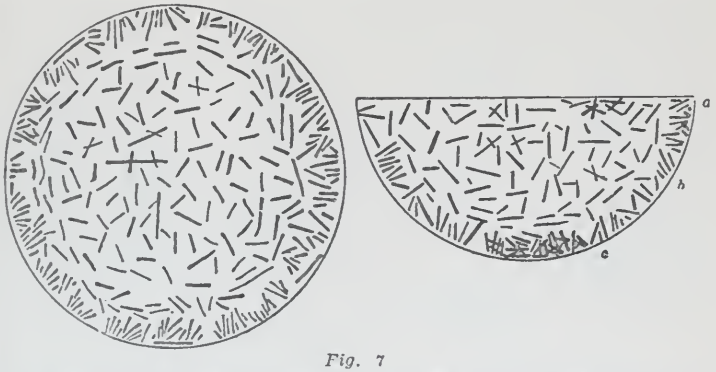



*Fig.* 7

(A) MICROSCOPIC FIELD, SHOWING THE TOP OF A HANGING DROP IN A NORMAL CULTURE OF TYPHOID BACILLI. (B) MICROSCOPIC FIELD, SHOWING A CROSS-SECTION OF THE DROP IN (A).

to a suspension of the microbe used to stimulate it, causes these microbes to gather together in little clumps or to agglutinate. This is the most specific test we have. Only the microbe used to stimulate the serum that agglutinates it, or a like microbe from any source, will be specifically agglutinated by this serum. This test has been proved to be practicable in detecting infections due to the typhoid bacillus and to a number of other microbes. As applied to the typhoid bacillus, it is known as the Widal test, so named after the one who first applied it practically.

In its entirety this test is also a very complex one. It is then called the absorption of agglutinins test. It is this complete test we mean when we say a microbe is specifically agglutinated. It is considered the ultimate test for determining the identity of microbes from different sources that look alike. For each microbe when added to its specific serum in sufficient numbers will absorb all of its specific agglutinins and none of those

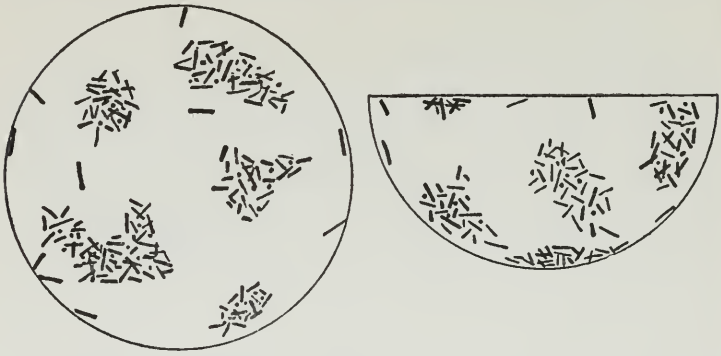


*Fig. 8*

(C) MICROSCOPIC FIELD, SHOWING THE TOP OF A DROP WITH THE TYPHOID CLUMPED OR AGGLUTINATED.

(D) MICROSCOPIC FIELD, SHOWING A CROSS-SECTION OF THE DROP IN (C).

stimulated by the injections of any unrelated microbe. So, if we put a sufficient number of a microbe whose identity we are not sure about into a serum that we know, by previous tests, contains the specific agglutinating substance for a known organism of the same appearance, and let it stand the required time at the right temperature, we get the serum free from the unidentified microbe by centrifuging, and put into it a suspension of the known organism; then, if the unidentified organism is the same kind of a germ as the known one, it will have absorbed all of the agglutinins

of the known organism that were in the serum and there will be no agglutination of the known organism, as there was before the unidentified organism had been allowed to remove the agglutinin. This is a very useful test to help determine the relationship of strains in an epidemic.

There is still another way known by which animals combat dangerous microbes. This is the most spectacular way of all and the one first discovered. We didn't describe it first, however, because the mechanism of its action is more complex than that of any other protective mechanism.

Circulating in the blood of all of us higher and lower animals are certain ameboid cells called white blood cells or leucocytes. These possess the power, under certain conditions, of seizing and drawing into their bodies certain of the microbes that have passed our first set of barriers, skin and mucous membranes, and gotten inside of our tissues.

The important part played by these wandering white blood cells in destroying certain microbes was first surmised by the Russian Metchnikoff while studying the digestive processes of the larvæ of starfish. He saw through his lens certain freely moving cells take up some colored pigment he had injected into the larvæ. He thought "if such cells can take up foreign matter, they can probably take up microbes too. And perhaps all animals are protected from disease germs in this way."

After many trials and exciting demonstrations he was able to convince other investigators that this kind of cell actually devoured some germs.

But at first his assumption was too inclusive. He be-

lieved that all harmful microbes were combated in this way and that this was the only way animals have of guarding against disease. And long and hot was the fight among investigators to prove that this destruction by phagocytes or cell destroyers, as Metchnikoff called them, was only one of the weapons animals have evolved to protect themselves against their germ foes.

Some investigators, as usual, went to the other extremes and thought that these phagocytes were only scavengers, taking up any foreign particles without selective action. But soon there were a host of research workers, chief among them Bordet, Wright and Neufeld, who showed that there is a certain substance circulating in the blood that attaches itself to the microbes, making it more easy for them to be engulfed and destroyed by the phagocytes. It seems to prepare the germ for being devoured. For this reason Wright gave this substance the name opsonin, from the Greek, meaning, "I prepare for."

An opsonin for a given microbe may become increased in amount in the blood of an animal that is recovering from an attack by the microbe, or that has been given regulated doses of a vaccine made of the microbe.

The resulting increase in the defensive power of the leucocytes is not as lasting as is the immunity produced against the other poisons of the microbes.

The question of why and how certain microbes, as well as other foreign substances, do not cause a decrease in the susceptibility of those who have been invaded by them or their products is one requiring so much technical knowledge to comprehend the little we know about

it that one can only say, as one has to say so often, we must leave it to the special investigators.

In these fragmentary sketches of how human beings and other higher animals react to attacks by harmful microbes, how a balance is reached between man and microbe, we have attempted to show that our body cells, on the whole, respond to the different poisons and other microbal products by manufacturing substances that neutralize these poisons and other products. These neutralizing substances are called antibodies; the poisons and other products stimulating the production of antibodies are called antigens.

Antigens of harmful germs that are injected into animals for the express purpose of stimulating the production of antibodies that may help cure or prevent disease are called vaccines, because they are used for the same purpose as was the first vaccine made from cowpox virus.

The chief reason why more people in these days are not impressed with possible dangers from pathogenic germs, why so many say "What is the use of vaccinating?" is that they forget that many people are immune to certain diseases because of the wide-spread vaccination that has been going on for years.

If we should stop the use of all vaccines and serums now; how much could we do to prevent infectious diseases by other hygienic methods alone? We can answer that if such hygienic methods were strictly carried out, most infectious diseases would be prevented. Why, then, you may ask, are not such measures employed? We answer, because it is a practical impossibility to carry out all of these measures.

Let us consider this. We know that microbes that are pathogenic for human beings may be conveyed through human or lower animal carriers, who may be normal, diseased or convalescent; we know that the germs may pass to another, directly, through fine droplets of sputum spluttered out from the mouth in talking or coughing, or from the nose in sneezing, or through the hands or other parts of the body contaminated with or containing the infectious germs; or they may pass indirectly by any utensils coming in contact with these infectious parts of the carrier. Food handlers in the picking, packing and transportation industries, jobber, fruit-stall and other human agencies contribute to the bacterial content. Then there are the dust and filth from flies and other vermin and lower animals. To prevent infection from such carriers means that all human beings must wear masks, that their hands and their excreta must be thoroughly sterilized; it means that in insect-borne diseases the insect carriers—mosquitos, flies, fleas, ticks—must be destroyed; it means that in diseases carried by other animals, like rabies transmitted by stray dogs, these animals must be destroyed.

One can realize after a moment's thought how impracticable—nay, impossible—it would be to carry out measures that would absolutely rid us of all sources of infection. The whole question of the use of hygienic measures, including the so-called sanitary procedures, is one of adjusting the measures needed to destroy all dangerous germs to the measures that can be practically applied.

There is no question but that many procedures that are practicable result at least in lessening the number

of dangerous microbes; and this is a worth-while thing to do, since the number of microbes is of importance in determining infection in many individuals. Those that have at least a little resisting power can take care of a few pathogenic germs.

Even if we could rid all carriers and their surroundings of all pathogenic germs, at a special time in a special neighborhood, would it be desirable? How would nature respond to this state of things? Antibodies would cease to be formed in these people. The people, or at any rate their descendants, might become fully susceptible to disease germs. If they went to another part of the world where such microbes still existed or if a carrier visited them they could be easily attacked.

There was a tragic illustration of this in the World War when troops came in from country places where they had not been exposed to measles. Epidemics of measles broke out in the various camps, attacking chiefly these country boys. They developed the disease in very severe form and many contracted a complicating pneumonia, resulting in a high mortality. We have had other striking examples in the American Indians and Eskimos when they have come in contact with diseases new to them, brought to them by people from the civilized world. Many have quickly succumbed to these diseases.

These tragic incidents show us that if we cannot have complete hygienic protective measures, and if we have no vaccines, we are bound to have more or less widespread fatal epidemics of certain diseases that we now prevent by vaccines. Notable among these are smallpox, diphtheria and typhoid fever.

We still have epidemics of influenza, measles, infan-

tile paralysis, whooping-cough, pneumonia and so on, chiefly because we have no efficient way of vaccinating against them or of otherwise preventing them. We either do not know the organism that causes them, or it is impossible to make or we have not yet learned how to make an efficient vaccine.

We see, then, that it is up to man to do everything he can to better all natural processes that promise to help him preserve and improve his life, to take his part in "nature's plan."

## CHAPTER V

## FAMILY RELATIONSHIPS OF MICROBES

As determined by human beings who know little about them, hence constant regrouping—"Family tree."

After all, from the human standpoint, we repeat there are only a comparatively few species among the microbes that are known to have a potent power for good or for evil upon man's life.

Where are these important ones placed in the three great classes described in the second chapter? How closely are they related? What traits have they in common? Have they any "poor relations" who may need to be considered? These are some of the questions we are now going to try to answer.

If we follow, as well as we are able, considering the minuteness of these creatures, the biologists' rule of grouping together, first, those that have similar forms and morphologic structure, drawing only a little on their physiologic activities, we find that these more important microbes fall into different groups or families among the great classes.

Students of biology know that the term "family" is used by classifiers to indicate a group of individuals that possess more traits in common than those classed as a tribe and less than those classed as an order. The word "order" is the name given to the first division under the broad division labeled class. The term "tribe"

indicates a group having more points in common than the group called genus (plural genera). Finally, the term "species" is used for the individuals making up the genera. We may give as an example the position of a well-known bacterium: Class—Bacteria or schizomycetes. Order—Eubacteriales. Family—Coccaceæ. Tribe—Streptococceæ. Genus—Streptococcus. Species—Streptococcus pyogenes.

Of course, as commonly used the import of the words "family" and "tribe" follows the custom of being quite different from what they had at their traced origin, so they have come to possess a number of different meanings; hence, qualifying words or introductory explanations must often be added to indicate what they may signify. For example, the human family, the mid-European family, the tribe of Judah, and so on. As applied to human beings to-day the term "family" may mean race, the term "tribe" kindred and the term genus family. Among botanists and zoölogists the terms family and tribe as used by the one group of scientists denote ranks slightly different from those used by the other, and the terminations used to designate these terms are different respectively.

Among the bacteria or lowest plants there are nine families. Among the molds or next higher plants there have been so many families described that we shall consider them here as the four groups that have commonly been recognized. Among the protozoa or lowest animals we shall describe likewise four large groups.

Some of these families seem to be closely related because of similar traits, though the individuals may not appear alike in shape and structure, and some individuals that look alike may exhibit such different traits

that they are placed at least in a different tribe or genus.

Then there are those mysterious invisible or ultramicroscopic viruses, in which we cannot yet distinguish individual forms, that can only be differentiated by certain physical and biochemical properties. They must still be studied as a group, though some of them may not be closely related in structure.

Of course, these groups are being actively studied by investigators throughout the world, and new facts and new relationships are being brought to light which may lead to some rearranging and renaming of the families, tribes and genera and of individuals in them. This has already happened a number of times. In fact, several changes have been made since we began writing this book. So the grouping or classification of these minute organisms is still in the depths of dispute.

To know just where to place each variety so that it falls into a significant group is often an impossibility with the amount of knowledge we possess. Even to do what little we are able with the aids we have requires so much painstaking study that few have had enough time or other means to carry on the required investigations. We are therefore still far from a satisfactory grouping of the individuals from the standpoint of the systematic biologist. As we have said, in order to show the relationships of these individuals so that they may be given a specific name and these species grouped as genera, we have had to draw largely upon physiologic and biochemical characteristics, since in such minute beings structural changes if they do accompany biochemical cannot readily be demonstrated.

Among other complications that make the grouping

of these minute organisms difficult or uncertain is the possibility—nay, the certainty in a number of forms, if not in all—of their changing traits that may have been used as a basis of grouping; that is, the progeny of certain families, while they may not change in visible form, may act differently under similar conditions because they have either lost old traits or taken on new ones. They usually lose qualities rather than acquire new ones. These fairly rapid changes take place in a comparatively short period of time from our standpoint, but one which allows thousands of generations of the microbe, which may pass through as many as thirty generations in a day.

The changed actions may be of extreme importance to man. Thus toxic bacteria may become non-toxic, virulent ones non-virulent, and many lose or change substances and characteristics that were of value in identifying them as well as of grouping them. Investigators have devised ingenious ways for studying these changing organisms.

In the first place, the students must be as sure as they can be that they are starting out with a pure culture from a biologic standpoint. So they begin by trying to isolate a single organism that they can see under the microscope. The ordinary way of getting something that is called a pure culture, the way by colony fishing that we described in the second chapter, is not considered accurate enough by these fishers of germs. But with all their care, and though the method they use—the capillary tube method, as it is called—is theoretically supposed to be the best method of getting a pure culture, it does not rule out clingers among the germs too small to be seen with our best magnification.

However, the chances are that they get a pure culture. At any rate they call it pure. And the earlier workers thought that under these conditions they had obtained a microbe upon which they could base predictions of future activity.

But alas! for their expectations. It was soon found that even these cultures, as we have said, do not always remain the same as they were when they were originally isolated. They split into those which develop colonies that are coarse or fine, rough or smooth, spreading or compact, mucoid or dry, and that have still other characteristics that are different from each other. Some lose or increase their toxicity or virulence, some lose their power to ferment certain sugars, some lose their power to agglutinate and to produce or absorb agglutinins. This last, as we have pointed out, is considered a very important method of species differentiation, and the most important if not the only test for determining epidemic strains.

All of these changes have been comparatively little studied yet, so we are still in a state of indecision as to their exact importance in classification and in other relationships in general.

We only know that they are making us more careful than ever in drawing deductions from observations carried on for short periods of time and with insufficient controls.

We see, then, that the so-called specific names of these organisms are liable to change and are therefore not so important to remember. Of course, they are of interest and importance to the biologist—as an aid in his studies, and to all of us as convenient if only temporary handles.

The family or group names are less liable to change and their common names are quite easy to remember. In this chapter we will list the family or group names, giving first the common names and then the specific names in parentheses following them.

The families among the bacteria that we are going to discuss are:

The coccus or berry-shaped family (Coccaceæ).
The nitrogen-using family (Nitrobacteriaceæ).
The family of bacilli without spores or the non-spore-bearing family (Bacteriaceæ).
The family of bacilli with spores or the spore-bearing family (Bacillaceæ).
The comma family (Spirillaceæ).
The coiled-hair family (Spirochæteceæ).
The false-branching family (Mycobacteriaceæ).
The true-branching family (Actinomycetaceæ).

The four groups among the yeasts and molds are:

The budding fungi or yeasts (Blastomyceteceæ).
The non-sepurate molds (Phycomyceteæ).
The septate molds (Mycomyceteceæ).
The imperfect molds (Fungi imperfecti).

The four groups or classes among the protozoa are:

The flagellates or forms moving by few hairs (Mastigophora).
The amœbas or forms moving by pseudopods (Rhizopoda).
The seed or spore-forming parasites (Sporozoa).
The ciliates or forms moving by many hairs (Infusoria).

Many of these families have grown so large that they have been separated into sub-families or tribes. Thus,

the coccus family has three tribes, known as Neissereæ, Streptococceæ and Micrococceæ. The nitrogen-using bacteria have two tribes, Nitrobacteriaceæ, or bacteria oxidizing nitrogen, and other simple elements, and Azotobacteria, or nitrogen-fixing tribe.

The family Bacteriaceæ is very large; it has eleven tribes. Only five of these are known to be of marked importance to man. One of the other tribes is of indirect importance, since it contains many members that are very pathogenic for plants; for example, the blight of pears and apples, the soft rots and black and yellow rots of melons, potatoes and beets, the blights of a host of other fruits and vegetables, the leaf spots in tobacco and other plants, and so on, are all caused by members of this bad tribe, called Erwiniæ. Many of the other tribes, too, are of great indirect importance, because they are soil bacteria that help in the great work of breaking down the organic material of dead plants and animals into the simple components that can be used again by living plants.

In one of the less directly important tribes is the historical bacillus that used to be called Bacillus prodigiosus; but now classifiers have decided that its right name is Serratia marcescens. This produces a blood red pigment, and was used earlier by some to deceive the superstitious in regard to what these deceivers claimed was a miraculous appearance of blood. It may produce red spots like drops of blood on stale moist bread, on boiled potato or on any moist organic matter. It may cause milk to become red, and may break up fat and cause rancidity of butter, but it is not poisonous for man. This bacillus, because of its small size and conspicuous growth, is often employed to detect de-

fects in the fine stone and porcelain filters that are used so frequently in the study of microbes.

Another bacillus in the same tribe is a very minute one that is found sometimes in the pus of abscesses and other purulent conditions. It produces a blue-green pigment with a pungent scent. Because of its easy detection by the color, it is also used in certain tests such as determining the efficiency of room disinfection. It used to be called Bacillus pyocyaneus, but now it is known among the purists as Pseudomonas æruginosa. It is only slightly pathogenic for man. This whole tribe is composed of individuals that produce beautiful pigments, red, yellow, violet, green, blue.

Now we come to the two tribes of this big family that are of greatest influence in man's life. The first two of these might be called the alimentary canal tribes, because they are found chiefly in this important part of man's body, some only in health, some only in disease, some in both health and disease. They are called Bacterieæ and Lactobacillieæ. In one of the other three tribes (Pasteurelleæ) is the famous Bacillus pestis, the cause of more fearful epidemics, spectacularly recounted in history and literature, than are the ravages of any other microbe. Of the two remaining tribes, in one, the blood loving tribe (Hemophileæ) are the misleading influenza bacillus and the irritating pertussis bacillus (whooping-cough bacillus); in the other is an encapsulated bacillus found quite frequently in colds. Whether it has much to do with them is another question.

The next family is composed of spore-bearing bacilli. It has two branches, and one is a big branch of aërobes or germs that grow in the presence of oxygen. Only

one of these is pathogenic for man, but this can be very dangerous, causing malignant pustule, septicemia and death. It is called Bacillus anthracis. The others of this branch are soil bacteria that help in decomposition. The second branch is composed of those that cannot grow in the presence of free oxygen; that is, they are anaërobes. Here we have a number of marked disturbers of man's peace. The lockjaw bacillus; the botulinus bacillus, that most famous of the food-poisoning bacteria; and a number of wound bacilli, causing infection of wounds with the development of gas in the tissues, followed by gangrene, are all found here.

The curved or comma-shaped family are chiefly water bacteria, though some are found in the intestinal canal. They may either appear as segments of spirals, the comma forms, or some may remain attached to each other, forming spirals like corkscrews or wire springs, when they look something like members of the next family. They are called vibrios or spirilla. Among these comma forms is only one pathogen, the dreaded cholera bacillus. Among the corkscrew varieties is one that all students know, because it is used for demonstration purposes. It is called Spirillum rubrum. It produces a beautiful red pigment in the depths of the medium.

The next family might be called the coiled-hair family, since its members were given the name Spirochetes because they look like coils of hair. As we said, they look something like some spirilla or the family we have just mentioned. On the other hand, some forms resemble certain flagellated forms among the protozoa. So some classifiers have considered them bacteria, others protozoa. Since the weight of evidence seems to be with

the former, we class them for the time being with the bacteria. To this family belongs the famous and notorious pale spirochete of syphilis, now called Treponema pallidum.

The next family is the pseudo-branching or false-branching family. Some authorities are willing to give its members the benefit of the doubt and place them among the true branchers, but what are taken for branches among them are so abortive, and appear only under such unusual conditions, that it is difficult to decide how to class them. In observing minutely the mode of growth of these forms the "branches" may be seen forming after partial division of an organism. At any rate, the claims put forward for classing them with the higher bacteria, like many of the claims of aspiring humans for belonging to the "first families," are not very strong.

The different groups included in this family by our latest classifiers are so unlike each other in many respects that each might be considered a tribe, if not a family, in itself.

Thus, there is the Indian-club-shaped group of which the diphtheria bacillus, the cause of diphtheria seen at its worst as membranous croup, is the chief. Then there is a group the members of which look a little like the diphtheria bacillus, but they can only grow when air is excluded; that is, they are anaërobic. Their effect on humans is not well understood, but they are supposed to cause a certain kind of ulcer occurring chiefly in the mouth. They are implicated also in certain gum abscesses.

Next there is the acid-fast group, of which that most insidious and deadly of all germs, the tubercle bacillus,

is the chief; and the ungrowable leprosy bacillus is a near relative.

Last there is the malleus bacillus, the cause of the glanders or farcy that occurs in lower animals and occasionally in man.

What a conglomeration of varieties to be placed in one family! Can you wonder that there have to be changes made, that some have to be moved to other quarters when we become better acquainted with them?

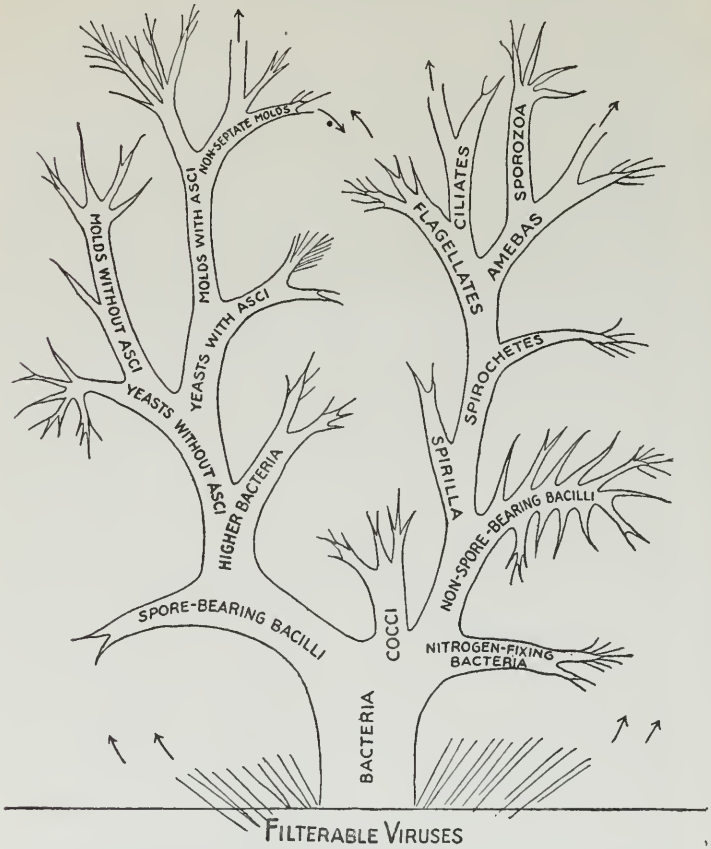
The next family, the Actinomycetaceæ, is composed of what have been called the true higher bacteria or the real branching forms. While they are like the bacteria in many respects, they are so like the molds in a number of particulars that they are classified with them by some investigators. Castellani in his latest classification includes among the molds not only these so-called higher bacteria but even those we have described under the common name, false-branching forms.

When we begin to consider describing in a book of this size the second great class of microbes, the molds, we are overwhelmed by the number of families and members of these families that have been reported, and the much and the little that has been written about them. A whole branch of biology, called mycology, has developed as a result of the studies of this group.

Because of the many differences of opinion in regard to their relationships we shall consider as belonging in this class only those forms that are called by Castellani and others the true yeasts and molds, and we shall describe the large commonly accepted groups rather than the many unsettled families.

First, there is the group called yeasts or yeast-like fungi. This group includes all those microbes that grow

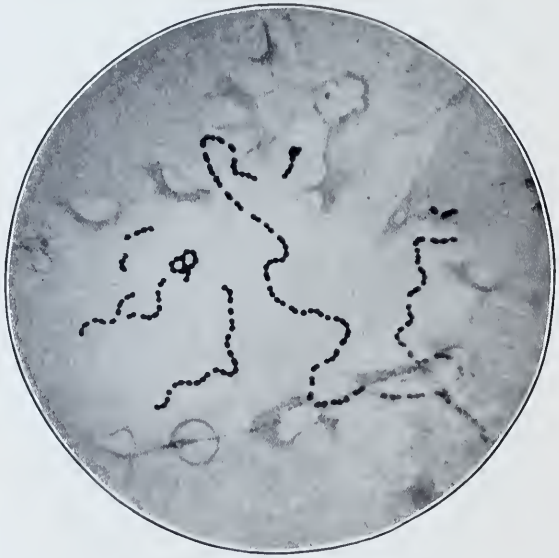
usually in the form of comparatively large oval or spheroidal cells that multiply chiefly by budding. Some




HYPOTHETIC TREE OF EVOLUTION OF MICROBES

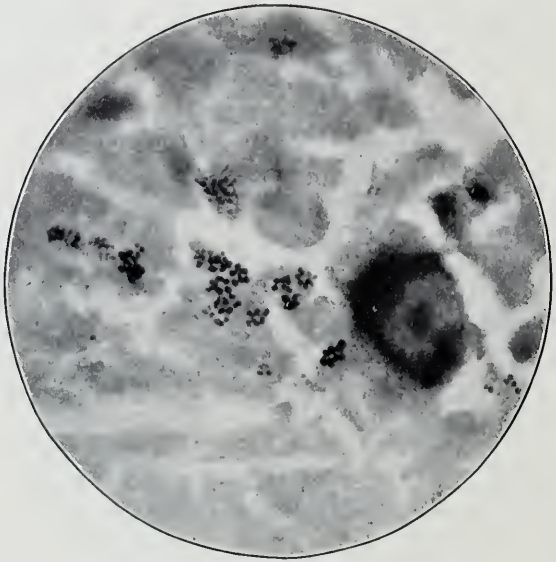
*Fig. 9*

varieties produce no mycelium or stem-like (thread-like) forms, except sometimes a few in cultures. Some

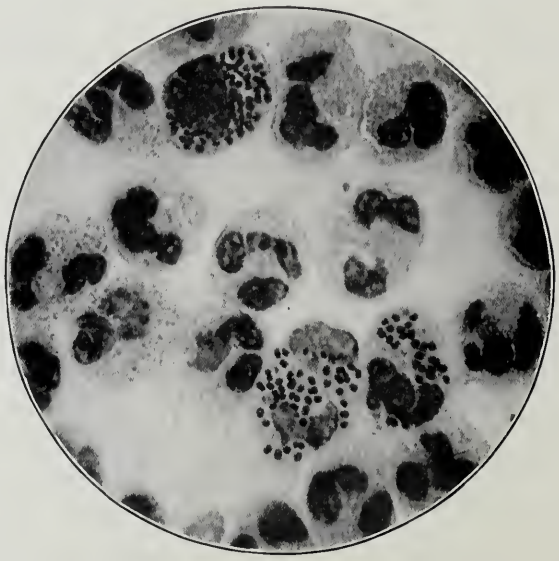


Cocci growing in chains. Hemolytic streptococci from blood poisoning. Enlarged 1000 diameters

Streptococci dissolving red blood cells. Hemolytic streptococci from scarlet fever. Natural size



Cocci growing in bunches like grapes. Staphylococcus aureus from an abscess. Enlarged 1000 diameters. (From Fraenkel & Pfeiffer.)



Cocci growing in pairs within pus cells, the gonococcus making a very distinctive appearance. Enlarged 1000 diameters. (From Fraenkel & Pfeiffer.)

varieties form cysts, called asci (meaning bag or sac), containing usually four spores or a multiple of four. In others no asci have been seen. The formation of an ascus has been chosen by classifiers as the chief morphologic trait for separating the yeasts and molds into two large groups (*See* "Tree").

These forms were called yeasts, from the Greek word which means to boil or seethe, because they were first seen in the foam and sediment of fermenting wines and beers. A number of varieties now classed in different families by the systemic biologists are commonly included in this group.

The big group of molds may be called the mycelial fungi, because the vast majority of them produce definite long-branching filaments or threads called mycelium. These filaments are usually much larger than the threads of the actinomyces, and they often have a double contour as seen through the microscope. In one group the filaments have no partitions; that is, they are non-segmented, except at certain stages in their life. This group goes by the name phycomycetes. In another group the mycelium is always segmented or septate; that is, it is made up of chains of cells. These were called mycomycetes, but now this group is split up by the classifiers. The fourth old group called the imperfect fungi contains all those forms in which a full life cycle has not been demonstrated. Among these are most of the forms found pathogenic in man. These too are now split up.[1] In the "Tree" the group proceeding from the "yeasts without asci" composes the Fungi imperfecti.

[1] See table in Castellani's book, "Fungi and Fungous Diseases," Am. Med. Assoc., Chicago, 1928.

The last class of microbes we consider, the protozoa, is also such a large group, and its classification is so unfinished that we can only describe its common division in general terms.

The flagellates have one to several delicate whip-like appendages which help them move about freely. The majority are harmless water microbes; most of them have a definite nucleus. Several have a modification of their body in the form of a fluted membrane attached by one edge and prolonged as the flagellum. Among these peculiar forms called trypanosomes are several enemies of man and beast.

The amœbæ, quoted as the type of man's beginning, are placed higher than the flagellates by some classifiers. They move about by pseudopods or false feet. Harmless, free living forms may be found wherever there are moisture and decaying vegetable matter. The matured individuals usually contain a single nucleus. This may divide into two or more, according to the stage of growth, and the kind of amœba. Then they may encyst into resistant forms that may live quiescent for many years. At least one form is known to be an active enemy of man.

The sporozoa are all tissue parasites that reproduce chiefly by forming many spores. That famous enemy, the malarial parasite, is placed in this group.

The ciliates, those forms that have many delicate hair-like appendages over their bodies, are mostly free living forms found in water. They have the most complex structure of any of the protozoa. With their double nuclear apparatus and their hairy striations they present a striking appearance. One only is pathogenic.

We have attempted to give some general idea of distinctive points characterizing the groups and families among the three great classes of microbes.

In the following pages we want to give the family or group history and the chief traits of the head or most important members of each family, tribe or group, and of those relatives that are considered worthy of being put in our blue book of microbes.

The question of the historic relationships of these groups of microbes, of their real origin, of their very beginning, is still unsolved. We call bacteria the lowest forms of life, but we do not know whether the other two classes sprang from them or whether these had a separate origin, or whether they all had their beginning in a form allied to one of the filterable viruses, or—we might keep on adding "ors" for some time without getting a clue to this so far impenetrable mystery.

An idea, right or wrong, may be obtained of the relationship between the different families and a parent stem, where the new type appeared and the "fixed types" remained, by glancing at the "tree" on page 80.

Of course, such a "tree" can only indicate the branches important to man, and like all attempts at graphic illustration it can only give a rough idea of possible relationships. Like all trees, pruning and grafting may help it grow more perfectly, or it may have to be chopped down.

The terminal lines indicate genera, and divisions between these and the main stem indicate tribes and families.

## CHAPTER VI

## THE COCCUS FAMILY

(Coccaceæ)

Pus producers—Blood invaders—Lung attackers—Brain membrane inflamers—Cheese makers.

The coccus family is one of the first three families recognized by the society of bacteriologists. Several of its members were accepted as being worthy of note between 1869 (Hellier) and 1879 (Pasteur). Even before this, minute globular forms, which were later identified as cocci, were seen by that keen-eyed observer Klebs, and after him by others in wound infections, though the final proof of their relationship to wound abscesses was not made until many years later. Because of their relationship to the formation of abscesses, boils and purulent lesions generally, the pathogenic members of this family have been called the pus-forming or pyogenic cocci. As they have become better known they have proved themselves to be a very important family and should be ranked high among the families of bacteria—high from the standpoint of their effects on man, but low from the standpoint of morphology.

It is only placed lowest by some classifiers in the grouping or classification of the bacteria because of its form—a coccus or berry shape. This spherical form is accepted by these classifiers as lowest, since it is assumed to be the simplest morphologic type.

From the standpoint of complexity of activities many of its members outrank most of the microbe world. So many-sided are the characters of some, so sudden and capricious their actions, harmless at times, harmful at others, that they might be called dangerously mischievous. When harmful they may attack almost any tissue in the body, but some have a way of becoming accustomed to a single tissue. Thus, some forms attack chiefly the skin, others the nerve tissue, others the mucous membrane of the throat, and so on.

They accomplish their purpose of injuring the tissue chiefly by penetrating and growing in it and breaking up its protoplasm by the activity of its ferments. Those germs that are killed by the body defenses are supposed to release a toxin as their dead bodies break up, a so-called endotoxin which, if it does not overwhelm the tissues, causes them to react and produce a complex antibody like one of those described in Chapter IV. Only recently it has been shown that some of the members form an exotoxin that acts chiefly on the skin and mucous membranes. Some make a poison that acts on the white blood cells or leucocytes. Some poison and destroy the red blood cells, producing the so-called laking of the blood through the dissolving or lysis of these cells, the hemolysis we mentioned in Chapter III. They have grown into such a large flourishing family that, as we have said, three tribes have been formed. Some members of each tribe have forced themselves particularly upon the notice of human beings because of their ubiquity and their irregularly harmful activity.

The first tribe, called micrococceæ, is composed of rounded forms growing singly or in pairs, groups or packets; it contains many non-pathogenic members

found in air and water. A few of its forms are harmful and harmless parasites in man. Most of them, as to staining, are Gram-positive.

The first member isolated in pure culture was obtained from a wound abscess by Rosenbach in 1883. It grows frequently in clumps like bunches of grapes, hence its name, Staphylococcus. It is the chief of the tribe.

Some of the progeny of this coccus develop a beautiful deep golden or orange pigment in their growth, others form a lemon-yellow color and others a pure white growth. These varieties (species) are called respectively the golden coccus or Staphylococcus aureus, the lemon-yellow coccus or Staphylococcus citreus, and the white coccus or Staphylococcus albus.

The golden coccus is the variety that is most harmful to man. Usually it is not at all dangerous. It grows as a parasite on the skin or on the mucous membranes of the throat of many healthy people without doing any harm. But if a wound occurs in the skin or mucous membrane, or a gland (fat or sweat gland) becomes plugged, lowering the resistance at that point, this microbe may start growing there and may cause that horrid pest, an abscess or boil.

Or if the person's susceptibility is increased for any reason, such as wrong diet, fatigue, exposure to heat or cold, and so on, a severe tonsillitis or other manifestation classed under the name "cold" may follow, with the growth of this organism becoming at least one of the factors in causing the whole clinical picture.

If the person in this condition is extremely susceptible to this microbe, or, in bacteriologic language, if he has no specific antibodies in his tissues, and if

the microbe itself has developed special powers of growing in these tissues and poisoning them, as some do, then the germ may rapidly overwhelm the individual, swarming in his blood stream, heart, lungs and all other tissues and quickly cause death. In such a case death is said to be due to blood poisoning or septicemia.

In all these cases the susceptibility of the human being is the more important factor; that is, this organism is not often so virulent in itself that it can cause serious epidemics by attacking several individuals in quick succession, though it may be fatal to the susceptible individual.

Among the cocci are two close relatives of the staphylococcus that might be mentioned here, more for their distinctive forms than because of their effect on man.

One grows frequently in fours, hence it is called tetragenus. It is found occasionally in abscesses accompanying the pyogenic cocci. It is frequently present in tuberculous sputum and has been found as the only microbe in an occasional case of conjunctivitis. It is supposed to have only feeble pathogenic powers, and is usually, if not always, only a secondary invader.

The other, called sarcina, is interesting because it grows in packets of eight or more cocci. It has been accused of doing a little harm to man, but the case against it has been dismissed through lack of evidence.

The next tribe to be recognized in this family has members that make a graceful, beautiful appearance when growing in liquid media, such as nutrient broth to which a little serum and sugar have been added. Their coccus forms remain more or less attached to each other as they grow, so they often present long chains

which are curved and twisted, hence the name of the tribe Strepto (which means twisted) and coccus, or streptococceæ. These chains look like strings of beads, and, stained by Gram's method, most of them stand out as Gram-positive blue or red globules, according to the stain used, on a contrasting background. They are not so strongly Gram-positive, however, as are the majority of the staphylococci. The streptococcus is more fastidious in its food requirements than the staphylococcus, growing more readily, as we have indicated above, when a little albuminous material and a pinch of sugar are added to the medium. Most species grow scarcely at all at room temperature. These need the body temperature for their best growth, and they like the human body to grow on.

It is frequently parasitic in human mouths—especially in the crypts of the tonsils—but it needs less encouragement even than the staphylococcus to grow through the human body and produce injury to its tissues. Depending upon the susceptibility of the person and the character of the strain of streptococci, some strains grow readily in the blood of their hosts, causing septicemia; some strains grow more readily through lymph spaces producing lymphangitis; some strains grow in the cellular tissues and lower layers of the skin, when they produce a condition called erysipelas, and so on.

It puts in an appearance in increased numbers so readily during the course of almost every contagious disease that it has often been incriminated as being the chief culprit in the tragedies of most of the infectious diseases, especially of those whose causes we have not yet found. Thus, smallpox, measles and infantile

paralysis, as well as diphtheria and other diseases whose causes are now known, have all at various times been said to be due to streptococci. Scarlet fever, of all the so-called specific exanthematous diseases laid primarily at the door of the streptococcus, is the only one in which a streptococcus has been proved to be its specific cause.

There are several facts that have led microbe hunters to accuse streptococci wrongfully of being the cause of the other diseases. First, the fact that they are so frequently present in all these other conditions; second, the difficulties our microbes have of establishing alibis when the deed is actually committed, when the disease really begins; and third, most important of all, the ability of the real destroyers to remain hidden. All this has made it very difficult to rule out these ubiquitous pushing microbes as being the real, the specific cause of the disease.

In some cases, notably in poliomyelitis and measles, they have not been absolutely ruled out yet. There is no question but that many varieties of streptococci are dangerous secondary invaders and often cause death when the original specific cause of the disease might not have done so. But we now know that they cannot cause specific diphtheria or smallpox; that they probably—at least any known forms—do not cause measles or poliomyelitis, and that certain forms do cause scarlet fever, erysipelas and puerperal fever.

The history of how these streptococci were finally proved to be the specific cause of the disease known clinically as scarlet fever is a record of one of the most —shall we say?—exasperating of all searches in pursuit of the elusive microbe. Clue after clue was found,

and proof was in sight several times but was not recognized by authorities. Research work of this type is well described by a remark of Haldane, published in a recent "Atlantic Monthly," concerning new facts discovered. He said that such discovery was usually based upon "years of very persistent and rather dull work in hundreds of laboratories," work that "had occupied far more time and probably required more thought and patience than the final stages."

Here is the story of the streptococcus and scarlet fever.

Almost as soon as streptococci were recognized as a distinct group among cocci, they were accused of causing scarlet fever. So many of these little chains of cocci were found in the throats of scarlet fever patients that the germ searchers thought these must surely be the cause of the disease. Then when similar chains were found in large numbers in many other diseases, some investigators said "they cannot be the cause." Others said, "But perhaps the streptococci in scarlet fever have special traits that we haven't discovered," and they proceeded to watch them very closely, and to try every test on them they could devise. And they published their discoveries, detailing lengthy experiments to show that they discovered a new trait that was possessed by the scarlet fever streptococcus alone.

But then along would come another group of research workers, who also published lengthy reports to show that these first workers had not used enough controls and that their test applied to many streptococci from sources other than scarlet-fever patients.

Among these numerous tests was one more important than the others, in that it divided the streptococci into

three large groups. Schottmüller (1903) found that certain streptococci produce a poison that destroys red blood cells by dissolving them completely (laking their essential hemoglobin), causing the so-called hemolysis we described in Chapter III. These cocci were called hemolytic streptococci. He found further that some streptococci produce a green color and only slight hemolysis in their growth in blood. These he called green streptococci. Others were found that produced no visible change when they grew on blood; these were called anhemolytic streptococci.

Investigators soon found that it was the hemolytic streptococci that occurred in such numbers in scarlet fever. But at the same time this kind was found in erysipelas, puerperal fever and several other diseases, so this test wouldn't work in separating them.

Then a Viennese physician, Moser, reported that the serum of horses that had been injected with hemolytic streptococci from scarlet fever agglutinated only streptococci from scarlet fever and no other streptococci, and that the patient's blood did the same. And he made a more important announcement than this. He said that his horse serum contained a specific antibody that cured most of his cases of scarlet fever.

Of course, detractors came along. They always do, especially at the beginning of an investigation. Aronson and others said that the horses they injected did not give a serum that had a curative effect, neither did it regularly agglutinate hemolytic streptococcus strains from scarlet fever.

Schick, who later devised that famous skin test used in combating diphtheria, pointed out that the reason people were getting such irregular results was because

not all horses respond equally to the injection of cocci. You had to choose your horse. This point we have found to be true for horses in response to all disease germs. But at the time not much attention was paid to Schick's observations.

In the meantime, every one was hunting for a method of demonstrating a toxin that might be produced by these hemolytic cocci and might be separated from them.

Then a Russian, Gabritschewsky (1907), reported that he could produce scarlet fever in children by a filtrate of his broth cultures of hemolytic streptococci from scarlet fever, and that the children after they recovered were immune to scarlet fever. A colleague of his, Savchenko, at the same time, showed that if he injected the toxic filtrate of his cultures into a horse and then the whole culture, he could get a serum that would cure scarlet fever.

But the world outside of Russia and Austria took no notice of these reports. And scarlet fever was still placed by the majority among the virus diseases. And oh, the searches that were made and the reports that were published on all sorts of false leads!

Then came the World War, that stopped all research on scarlet fever as well as on most problems not concerned directly with the winning of the war.

After that another group of workers opened up the question of the specific agglutination of the strains of hemolytic streptococci from scarlet fever and claimed anew that practically all these strains fall into one agglutination group.

Opposers to this arose as they did before, this time including the authors, who showed that this did not

hold when large numbers of strains of these streptococci were studied. We showed that there were at least four agglutination groups or types. This fact, of course, did not detract from the claim that hemolytic streptococci are the cause of scarlet fever, because we know that among other kinds of bacteria one may cause a definite disease, and yet among the strains causing that disease there may be several agglutination types of the bacterium.

Drs. George and Gladys Dick of Chicago, in the meantime, had shown that scarlet fever had followed the implantation of strains of hemolytic streptococci from scarlet fever into the throats of human volunteers. Thus, they added strong evidence to that given by an earlier observation of ours that scarlet fever followed the accidental swallowing by one of our laboratory workers of a small amount of culture of a hemolytic streptococcus from scarlet fever. Just at this time Schultz and Charleton demonstrated that convalescent scarlet fever serum produced blanching or fading of the rash when injected into the skin of a scarlet fever patient in the region of the rash. And Dochez in New York showed that the serum of a horse injected with scarlet fever streptococcus in such a way that its toxin would act throughout the horses would produce blanching of the rash in patients in the same way as would convalescent serum.

Then came the crowning piece of evidence.

The Drs. Dick demonstrated by a new application of an old test, the skin test discovered by Schick, that the hemolytic streptococci from scarlet fever without doubt manufactured an exotoxin that gave a positive reaction, a little red spot, when a drop of a diluted

solution was injected into the skin of a person susceptible to scarlet fever.

For a wonder, nearly everybody agreed that this test works. Our minds had been prepared by the universal use of the Schick test. Then we all proceeded to show that such a toxin could stimulate the production of an antitoxin that seemed to work miracles in curing scarlet fever. And thus, at last, the work of the Viennese and Russian doctors and the other early researchers was vindicated. But—there always seems to be a but in this research work—but the close relationship between all of the harmful strains of hemolytic streptococci is still under investigation, and the end looks far away. The reference list of these studies occupies many pages of our indexes.

The other large group of streptococci, those that produce a green color when growing on blood agar plates—the so-called green streptococci—are met with in both disease and health even more frequently than are the hemolytic streptococci.

Many of them are strong lactic acid producers, and several are used as commercial starters and ripeners in the manufacture of cheeses. As to the harmful varieties, there is no question but that some are instrumental in causing certain cases of heart disease, subacute and acute forms of rheumatism and septicemia.

Then there is the most noted member known of this tribe, whose name alone is enough to send chills down one's spine—the pneumococcus, the most common cause of all pneumonias, and the only cause of the most dangerous type. Ah, the tragedies this coccus has incited and is still inciting, the sudden attacks, the horror of gasping breath, then death within a week!

No wonder certain wealthy people are providing funds for carrying on more investigations concerning the different varieties of pneumococci, the kind of antibodies they stimulate, and, most of all, how to make these antibodies or serums so pure and strong that they will have a quick curative effect; for they must act very quickly to be effective in this disease.

The pneumococcus, like so many of the microbes that may be pathogenic for humans, has complicated its relationships to man by having a number of types each of which stimulates in any susceptible animal the production of antibodies that act only on their own type of coccus.

You may ask, "Why not have a mixture of the antibodies?" That is what we are trying to get. But our endeavors are further complicated by several things. First, there are a number of pneumonia cases due to pneumococci that do not fall into any of the types that are affected by the antibodies we have so far produced, and we don't want to give serum unnecessarily.

We are classifying now the pneumococci of all of our pneumonia cases that do not respond to the type serums we already have, in order to find new types. Miss Cooper, in our laboratories, has just found several new types and is starting to produce antibodies to combat them. It would be impossible, of course, to treat every case with the right kind of serum. So many different varieties of pneumococci exist that one cannot hope to have a completely polyvalent antibody solution that will cover all.

The second difficulty is that not all the types of pneumococci produce antibodies of equal potency. The very ones that cause the highest percentage of deaths

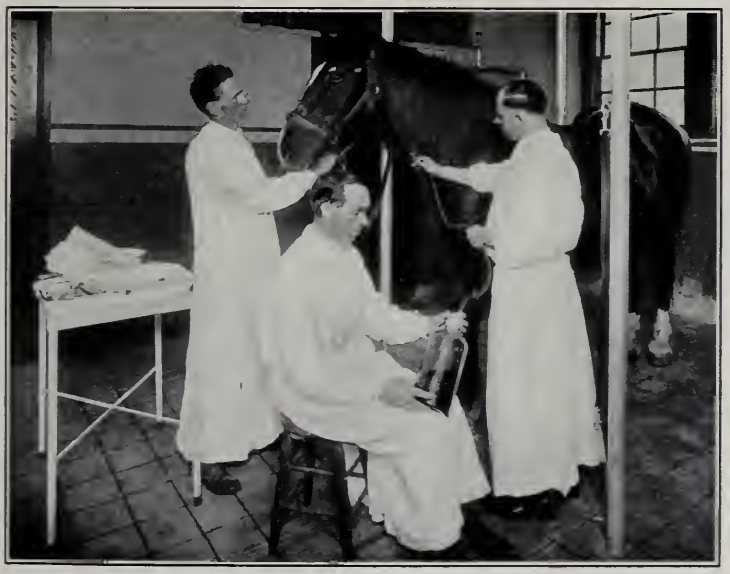
in those attacked produce the weakest antibodies. We are trying every means we can devise to make antibodies stronger. We have partly succeeded. Fortunately, the type that stimulates the best serum is the one that occurs most frequently in this country.

Leading from this is another difficulty, which you may already have thought of. This is that if we want the best effects of the strongest serum, we must know the type of the pneumococcus that is causing that particular case of pneumonia. In order to find this out the sputum has to be sent to the laboratory to be subjected to a series of tests.

And here we meet with the several difficulties of identification. For this coccus also has a number of forms that are not pathogenic for humans or other animals, and as these are frequently parasitic in throats of healthy people and might be growing along with others, they must be excluded. Indeed, this coccus was introduced to us first, as were a number of our microbes, while living in a normal human being—this time in the sputum. Dr. Sternberg found it in 1880 while examining his own sputum. Then he examined, as a control for some investigations on malaria, the saliva of a number of healthy students and found similar cocci in several of the samples. Of course, the pneumococci he found were growing as parasites in the mouths of these humans, but they had potential pathogenicity.

The chief morphologic mark of identification of these cocci as a genus is the formation of a mantle or capsule about them. A second clear-cut point of generic distinction is that bile dissolves them. But in order

Injecting pneumonia sputum into the belly of a white mouse to help determine type of pneumococcus



Drawing blood from horse that contains pneumococcus antibodies

Filtering after precipitation

Filtering through paper pulp

to tell which type of serum must be used in any given case we must first get our pneumococcus in pure cultures, which we do by injecting the saliva into the belly of a mouse, where the coccus grows so quickly that we can recover a practically pure culture of it in a few hours. Then we use the agglutination or precipitation test. All this takes time. So we give our patient a dose of our polyvalent serum first, and as soon as we find out which type of coccus is attacking him we change to that type of serum if we have been able to produce one that is potent.

Now we come to the members of the coccus family that have been placed in the third tribe. This tribe is named after the man Neisser, who first saw the chief of the tribe. Adding to his name, as usual, the Latin termination used to indicate tribe, we have the word Neissereæ as the name of this tribe. There are only two members that are important to man. These are strict parasites and have an insidious pathogenic action.

They have a few near relatives, who are of no great importance, except sometimes, as may happen in all families, to interfere with the identification of the important ones.

The chief of this tribe was first seen by Neisser (1870) long before it was isolated in pure culture by Bumm (1885). It is a distinctive looking coccus, one of the few that can be recognized under the microscope when taken directly from the individual affected. It almost always grows in pairs, each elliptical with flattened side closely apposing the other. For this reason it is often called the biscuit-shaped coccus. These pairs are more or less equally spaced from each other

when they grow in their host's cells. When stained with a double stain the pairs show up very well. They are decolorized by Gram's method of staining.

This coccus is extremely fastidious in its requirements for growth. It is one of the most capricious of microbes. It must be planted on very special media at frequent intervals which must be quite regular. Furthermore, the temperature at which it is growing must not materially vary.

Neisser found this coccus in the pus cells of discharges from humans suffering from a disease called gonorrhea because it affects so often the genital tract. For this reason Neisser called this germ gonococcus. Its latest scientific name is Neisseria gonorrhœæ. So far as we know, it grows only in human beings and is practically only transferred by direct contact. It dies very quickly outside of the body. While it chooses the genito-urinary tract for its chief dwelling-place, it has a second site of election in the lining of eyes, especially of new-born infants. It was the chief cause of infantile blindness before the use of silver nitrate solution dropped into the eyes of the new-born babies.

In those early days both nurses and doctors occasionally contracted a fatal blindness through infection with this germ. This occasionally happens yet to the careless. It sometimes passes into the general blood stream and causes severe arthritis or inflammation of the joints. It attacks the hosts, as do others of this family, by producing a poison in its body, an endotoxin, that is released on its death. It grows in the epithelial cells of the mucous membrane of its host, and causes by its irritation the accumulation of the white cells which may take up many of the cocci, so that some

cells may be full of the characteristic, spaced, biscuit-shaped cocci. It is because of this appearance that we can make a microscopic diagnosis of the disease in a short time.

This organism has come into great disrepute, but its hosts are often innocent victims. The disease it causes is classed as one of the two great venereal diseases. The other is syphilis, whose cause belongs in another family which will be introduced later.

The second member of this tribe looks very much like its chief, but its individuals when growing in pairs are more spherical than elliptical, and one of them is apt to be a little larger than the other and to stain more faintly. It, too, readily gives up this stain by the Gram's method. It is fastidious in its food needs, but grows a little more easily than does the gonococcus. It likes the nerve tissue membranes (meninges) to grow on. It also likes the back of noses and throats (nasopharynx) to hibernate in. There it stays growing slowly in the occasional carriers, and when it gets to a person who is susceptible it passes into the linings of the brain and spinal cord and grows there, causing a purulent inflammation. It is called meningococcus, or, according to the latest classification, Neisseria intracellularis.

There seems to be much natural immunity to it, since few cases occur. But once in a while there is a small epidemic, or occasionally quite a large one. It has never been found in lower animals, so the occasional human carrier must be the starter.

Since it grows over the membranes of the brain and cord it is hard to reach for treatment, and for years many of the people attacked died. Then Jochmann in Europe and Flexner in our country found that if the

serum of animals fully immunized by the cocci was injected very directly into the spinal canal of those attacked many more recovered than those not so treated.

In this tribe, too, the individual strains vary in their faculty of stimulating the production of antibodies, and we have had difficulties in getting serums that will have equal power to cure in these cases.

We have no clear-cut test to show the power of this serum, as we have with the diphtheria antitoxin, so we have to rely upon several potential tests which do not always give us results that we can rely upon.

In giving this serum to a patient we make tests to find out if he is suffering from meningococcus infection. The spinal fluid that is drawn off is submitted to a number of examinations in the laboratory, including the hunting for the meningococcus.

## CHAPTER VII

## THE NITROGEN-USING FAMILY

Soil microbes—Life-giving forms in the life cycle of plants and animals—Oxidizers of simple chemical combinations.

We have already pointed out the known fact that all bacteria, as well as other forms of life, use nitrogen to supply a fundamental need in their growth. The bacteria of the nitrogen-using family, which make up perhaps the most important part of the microbic population of the soil, are noted for using nitrogen in its simplest combinations. More striking still, some of them use and fix atmospheric nitrogen directly, thus performing, merely by simply growing, an act that man has only been able to accomplish after many attempts and through the use of very powerful physico-chemical agents and electric machinery.

The nitrogen-fixing bacteria have also been called the life-giving bacteria par excellence. And these marvels among microbes justify this thrillingly significant name. Without bacteria of this type, existence of living forms on our earth as we know it would cease. From the beginning of time these chemists of the soil (as all soil microbes are called that take part in the life cycle of growth of plants and animals) have probably been utilizing their chemical powers, some to oxidize ammonium compounds to nitrites, others to oxidize nitrites to nitrates ready for plant absorption, and still others,

perhaps the most important, certainly the most surprising of all, have been actively engaged in absorbing atmospheric nitrogen and making it a part of their protoplasm—fixing it—thus placing it in such chemical combination that it can take part in the life cycle again.

This last group of microbes, therefore, stops a so-called leak of nature, the loss of nitrogen from the soil to the atmosphere as inert nitrogen gas, after the complete breaking down of nitrogenous material through its various stages into nitrogen and water. This "leak" would be disastrous to living forms if it could not be stopped or if there were no way of making this inert atmospheric nitrogen again available for plant food. This is the very thing that nitrogen-fixing bacteria do. Some use the nitrogen directly as they grow freely in the soil and others as they grow in certain plant roots.

It had long been known that certain plants belonging to the legume family, such as peas, beans and clover, have along their rootlets little nodules which were first thought to be diseased growths like galls (Malpighi in 1687) on other plants. Then it was observed that the plants that showed these nodules grew larger and better in every way than did those that had no such tubercles, and that the soil that grew these plants and had them plowed into it seemed richer than soils that used other "green manure." This led the farmers to use clover on their farms to help restore the fertility of the soils. But it was not until the latter part of the nineteenth century that the rôle played by these root bacteria in restoring nitrogen to the soils in usable combinations was fully demonstrated.

It was first shown that in sterilized soil no plants finally grew. Then it was shown that if leached water from soil that was growing legumes well was poured over this sterilized soil before all the plants transplanted to it were dead, the dying legumes recovered and grew well, but other plants did not. Along with the growth of the legumes appeared the little nodules. Then the little rodlets and ovoid bodies which filled the nodules and which had already been seen by several observers began to be studied more closely. In 1888 a German, Beyerinck, obtained a pure growth of these, and Prazmovski, in Russia, and other investigators in different parts of the world showed how the organisms enter the root hairs of the young plants, pass through the cell walls, stimulate the formation of nodules and cause the whole plant to grow vigorously. It is most interesting to follow the progress of these investigations step by step and see how minutely the work had to be controlled or checked up at each step to rule out error and false deductions. The relation of the organisms to the plant in which they grew was shown to be a true symbiosis—that is, a growth where each helps and is necessary to the other. It was found later that there were several varieties of these nodule bacteria, some better for certain legumes and others for other legumes.

In the meantime Winogradsky, a Russian, had found an anaërobic bacillus, or one growing without air, that had quite marked powers to fix atmospheric nitrogen directly. This is a spore-bearing bacillus that belongs in another family. It goes by the name Clostridium pasteurianum, or Clostridium butyricum. We will speak of its other uses later.

Then Beyerinck found a large group of aërobic bacteria in our nitrogen family that could also fix atmospheric nitrogen directly.

When these bacteria were grown in a culture medium containing only water, sugar and some mineral salts but no nitrogen it was found that, as they grew, nitrogen was demonstrated in such cultures in amounts corresponding to the loss of nitrogen in the measured quantity of air in which they grew.

It has been found since then that the ability to fix atmospheric nitrogen is possessed in some degree by a number of species of bacteria and by certain molds, but that the two groups just mentioned, especially the latter, are the chief direct nitrogen fixers.

While the fixing of free nitrogen in such a chemical combination that it can be used by plants for food is an important and dramatic act, other members of this family have different powers over nitrogen in combination that produce equally important, if not such dramatic, actions in carrying on the cycle of life on our globe. We have already mentioned them. They are the bacteria that oxidize ammonia or ammonium compounds into nitrites and others that oxidize nitrites into nitrates, ready for absorption through plant roots.

Pasteur (1862) was the first who suggested that microbes play a part in the production of nitrates in the soil, but it was not until about 1880 that the mystery of the cause of nitrate production in soils was cleared up. It was found (first by Schloesing and Müntz) that when dilute solutions of ammonia were passed slowly through long tubes filled with soil, the leachings contained nitrates in amount corresponding practically to the quantity of ammonia used. If this

soil in the tube was first sterilized, by heating or by an innocuous chemical germicide, the ammonia passed through unchanged. Then if to this sterilized soil was added a little unsterilized soil, the leachings again contained nitrates. This was proof that there was something in the soil that was destroyed by heat, or a chemical germicide that changed ammonia into nitrates.

The investigators concluded that microbes were the things to hunt for, so they isolated in pure cultures various bacteria, yeasts and molds that were in the soil, and added each to the sterile soil in the tube, but the ammonia passed through unchanged.

For a dozen years many more than a dozen microbe hunters tried to find a single kind of microbe that would cause nitrates to come out of the tube in the leachings.

At last, Winogradsky was able to find out why they had all been unsuccessful in their search. They had been using the wrong kind of culture media. He showed that these special bacteria would not grow on any kind of the common nutrient culture media.

Instead of the comparatively rich nutrient gelatine medium that the others had used, he used a silica jelly containing only a few inorganic salts. He found that there were two bacteria that made the change, one in each of two stages. The bacteria of the first stage, that is, those that oxidized ammonia to nitrites according to the formula $NH_3 + 3O = HNO_2 + H_2O$ might be called nitrous or nitrite bacteria. Winogradsky gave them the name nitrosomonas.

The bacteria causing the second stage of oxidizing nitrous acid or nitrites to nitrates—

$$HNO_2 + O = HNO_3$$

—known as nitric or nitrate bacteria, were called by Winogradsky, nitrobacter and nitrosococcus.

A few other bacteria have been described that change ammonia to nitrates, one even that oxidizes it directly to nitrates, but the greater part of the work is done by the species discovered by Winogradsky.

In order that these organisms may do their work of oxidizing thoroughly, the soil must be in just the right condition. It must have plenty of oxygen, the right temperature and not too much moisture or organic matter, the right reaction to alkalinity (magnesium lime should be added if necessary), certain mineral salts, and no deleterious bacteria or chemicals present.

When ammonium compounds have been converted into nitrates we are at a point where we may conveniently start describing the important events in the nitrogen life cycle that joins plants with animals.

We may start at the stage we have just mentioned, in which the nitrates are absorbed from the soil through the roots of plants. These help to form proteins in the green leaves of the plants, aided by sunlight and chlorophyl. These two agents, chlorophyl and sunlight, transform the simple nitrates, carbon dioxide and water into living matter.

In the second stage, plant proteins are eaten by animals and in part help form animal proteins, in part are broken up into urea, and in part pass out as other nitrogenous products which are unsuited for animals and too complex to be used in this form by plants. In the third stage, the urea and the other nitrogenous products discharged by living animals or locked up in dead animals or plants are broken up by the bacteria of decomposition into ammonia or its compounds and water.

Some nitrogen is also set free at this stage, forming the "leak" spoken of before. This process is called denitrification. Other products of decomposition, such as carbonates and sulphates, are formed. Many bacteria, in fact members from each family of microbes, may take part in this complicated process of decay or rotting.

In the fourth stage, ammonia is converted by the nitrifying bacteria, as we described above, into nitrites and nitrates.

Thus we must have decay to provide food for our plants before we can have life. The organic matter in the soil must be split up or changed into simple compounds before it can be used by the higher plants, the carbon returned to the air as carbon dioxide to be used by leaves, the nitrogen to the soil as nitrates to be used by roots, and a number of minor but equally necessary changes, reactions and interactions, such as the oxygen supply to the soil affecting the formation of carbon dioxide, the supply of minerals necessary for the growth of various forms, phosphorus, sulphur, iron, calcium, zinc, manganese, potassium and magnesium.

Micro-organisms take part, though not as directly or regularly as they do in the nitrogen cycle, in changing all of these other elements from their combinations in the tissues of plants and animals where they are unavailable for plant food. Thus we may speak of a carbon cycle, an oxygen cycle, a phosphorus cycle, a sulphur cycle and so on. And all of these vary according to many different factors in the soil. There is even a group of these microbes the members of which are capable of breaking up cellulose, the dense wood of trees. Winogradsky found that by altering his silica culture medium a little he could obtain an almost pure

culture of these microbes directly, just as he was able, by other slight changes to get luxuriant growths of different species that perform other marvels of destruction and construction.

It is little wonder that the study of soils has been carried on so intensively, that the research workers in agricultural stations devote a large part of their time to the study of soil micro-organisms and their effects upon crops.

In view of the fact that there are so many different kinds of microbes competing with each other in the use of the environment of the soil for their growth, it is little wonder too that agricultural research workers have been on the lookout for helpful microbes that destroy harmful ones, and harmful ones that interfere with helpful ones. We have only to read the reports from the Department of Agriculture to realize how far we still are from controlling plant diseases due to microbes and how great are the losses to farm crops from such diseases.

The complex nature of the soil and the important results that may be obtained from the ability to regulate and control its physico-chemical evolution and its microbal population make it a fascinating subject for study.

The modern agriculturist must be well versed in soil and plant bacteriology as well as in a number of other sciences if he is to take advantage of all the conditions that may produce better crops.

By some classifiers this family is placed first in the system of classification, because its members are able to live on the simplest of foods; but since the adult forms of their individuals are bacilli or rod-shaped

bodies, they must come after the cocci if we accept the coccus as the simplest morphologic type.

Here we see again that the vexed question of classification leaves us more or less confused. There is no confusion or uncertainty, however, about the knowledge we have gained of the importance of the activities of members of this family in the lives of the human family. They certainly have been and are our friends.

A group of bacteria that perform a very individual service for man is placed as a genus among the oxidizers in this family. The members of this group oxidize alcohol to form acetic acid or vinegar. They are appropriately called the acetic acid bacteria. Their generic name is acetobacter. They are an indispensable part in the industry of manufacturing vinegar. The scum called "mother of vinegar" that is often formed in the vinegar used in the home is made up of these acetic acid bacteria. If a little of this "mother of vinegar" is added to the home cider, and plenty of air is given it to furnish the oxygen needed for oxidizing the alcohol in the cider, the cider may change into vinegar in a comparatively short time.

In the manufacturing of vinegar on a large scale a device is used whereby the "mother of vinegar" is spread over a large surface with maximum exposure to air. A heap of shavings gives such a surface. Then diluted alcohol is sprayed over this bacteria-covered heap, and the bacteria get busy with their taking of oxygen from the air and their adding of it to the alcohol as indicated in the equation

$$CH_3CH_2OH + O_3 = CH_3COOH + H_2O.$$

## CHAPTER VIII

## MICROBES LIVING IN THE INTESTINES

The "long-life" microbe—The signal bacillus—The food-poisoning group—The typhoid-dysentery group.

The intestinal canal of man is a great gathering place for microbes. Several kinds are always found here, some species frequently and others seldom. Some pass with the digesting food quickly through the coiled length of the intestines, others linger by the velvety corrugated walls of this long winding passage, finding ideal conditions for growth in its warmth, food, moisture and reaction. Some are helpful to their host by producing substances that are harmful to other more deleterious microbes; some are indifferent; a few, and fortunately these are among those that are seldom present, are harmful. Among these kinds all of the great classes of microbes may be represented, but the bacteria constitute the bulk of them; indeed, these, as a result of their vigorous growth, form a large part of the dejected fecal masses. It is estimated by dry weight that from five to eight grams of bacteria, living and dead, pass daily out of the intestinal canal. This means about a hundred trillion bacteria. Such a large number can scarcely be realized, but their estimation by bacteriologic methods is comparatively simple.

A definite quantity of the feces is shaken up in sterile salt water and from this a series of dilutions is made

until one is obtained which is thin enough to show the microbes well separated from each other, when a measured amount, as, for instance, one hundredth of a cubic centimeter, is spread uniformly over a known area marked on a glass slide. The film is dried, covered with a thin solution of gelatine, fixed in methyl alcohol and stained with a staining solution that differentiates the microbes from the detritus. Then the germs are counted under the microscope, their number multiplied by the dilution used and their whole number estimated.

The regular port of entry of these teeming trillions is the mouth. At birth the alimentary canal of a normal baby contains no germs. A few hours later they begin to appear, mostly entering through the mouth, a few growing up through the anus. The chief source is the food and the drink. The microbes, however, don't enter in trillions. The food and drink of infants usually contain, or should contain, few germs. Adults, according to Thom, may take in many germs on uncooked food such as fruit, even though it is fresh and sound. After the microbes have passed the defenses of the stomach with its acid gastric juice, and of the upper intestine with its slightly germicidal secretions, the varieties that find conditions good for them in that natural incubator, the large intestine, make great strides in growth and soon dominate any others that may have survived; so while many kinds may die, the few that survive go on increasing to the enormous numbers given.

What effects have these moving masses of microbes on man? Are they necessary to him in digesting his food or in any other way? Or could he live without them? Many research workers have busied themselves with trying to answer these questions by studies on lower

animals. After numerous trials a few experimenters have succeeded in getting eggs of lower animals free from microbes—eggs of cockroaches, of tadpoles, of chickens, of turtles. Then the investigators tried to incubate the eggs and hatch and rear the young in sterilized incubators, and feed them with germ-free food. A few of these trials proved that it was possible to rear these animals with germ-free intestines. Baby guinea-pigs and goats also have been obtained free from germs (by Cæsarean section) and reared in germ-free surroundings. These have lived long enough to show that animals may live without microbes, and some interesting observations have been made on them. But since the practical impossibility of ridding our surroundings of these ubiquitous mischief-makers is accepted as a fact, the next question investigators attempted to solve was, Can man control the bacterial flora of the intestines?

It had been observed that the first predominating microbe in a normal baby's intestines is a kind that produces large quantities of lactic acid. Milk, which is the baby's chief food, contains much lactose or milk sugar, and these bacteria use this sugar as they grow, breaking it up into lactic acid. It was found that certain types of organisms cannot grow in an acid medium, especially the so-called putrefactive bacteria that are supposed to help produce that much-discussed condition called "auto-intoxication." Certain distinctly pathogenic germs also do not grow well in an acid medium. Many studies have been made, therefore, with the object of finding out ways of transforming intestinal flora by implanting a helpful microbe of the lactic

acid type, with the hope of correcting certain types of intestinal disturbances due to the putrefactive bacteria.

There are many varieties of lactic acid bacteria. The kind that appears first in the intestines of babies fed with mother's milk, known as Bacterioides bifidus, does not grow there so readily as the child becomes older. In the search for kinds that will grow readily and remain at home in the intestines supplanting other forms, Metchnikoff called attention to the fact that Bulgarians are a long-lived people and that a common article among their foods is a sour milk. They use as a starter for coagulating their milk a certain lactic acid bacterium that came originally from "yoghurt." Metchnikoff obtained pure cultures of this starter, and the laboratories throughout the world obtained subcultures of it and recommended it for trial to those who suffer from intestinal putrefaction. But alas! those who took this culture continued to suffer from "indigestion." Lactobacillus bulgaricus, as it is called, while it grows well in cow's milk and mare's milk, will not grow to any extent in the human intestinal canal. It will not supplant the putrefactive bacteria there. Thus this "bacillus of long life," as it was called, has lost its reputation. But the experimenters didn't give up the search. They proceeded to study the lactic-acid-producing bacilli found in normal human beings. They discovered that one kind called now Lactobacillus acidophilus produces more lactic acid than does any other bacillus found in the intestinal canal of man. This has seemed to work well in a number of cases of human auto-intoxication. Cultures are supplied in milk and also in compressed tabloid forms covered with chocolate. Though these

tablets are very pleasant to the taste, they are not considered as effective as the acidophilus milk. The patient should be put on a minimum protein diet. There is some doubt as to whether a true implantation occurs. It usually takes time, many of the bacteria and much lactose or milk sugar for an apparently successful implantation. The great majority pass through the intestinal tract and are voided. That Lactobacillus acidophilus is a helpful microbe, however, in some cases, seems reasonably certain. Several different kinds of energetic lactic acid producers are used in industries for the making of sour milk products, but none of the cultures have been successfully implanted in humans. The milk in this form, however, may be more easily digested by some people.

Very little is known about how much some of the other microbes that are usually found in the intestinal canal help keep the balance of normal microbic growth there, but for the most part the others seem to be quite indifferent to man's welfare, except on those few occasions when one takes on a slightly virulent quality. One of the varieties, however, called the colon bacillus, or B. coli, a well-known member of the tribe bactereæ, that is always present and usually in very large numbers, is of use to man indirectly in this way. It is easily identified by a special test, and since its presence is a sign of fecal contamination, this test is applied in the examination of water, milk and oysters to indicate whether these substances have been contaminated in any way by sewage. This test is called the presumptive test, and the bacillus might well be called the signal bacillus.

This bacillus was discovered in the stools of children by a German named Escherich, as far back as 1886. As

many different relatives of it have been found, in both man and lower animals, they have been grouped under the generic name Escherichia, derived from the name of the discoverer, so now this ubiquitous bacillus, this B. coli, has specifically the high sounding name Escherichia coli. This bacillus has about the same powers of resistance to germicides as has its dangerous relative the typhoid bacillus, hence it is used in testing the strength of germicides against that type of germ when one does not want to take the risk of using the typhoid bacillus itself.

B. coli, like all of this tribe, is a short Gram-negative rod that moves irregularly through a liquid medium. If a little sugar, almost any kind except saccharose, or cane-sugar, is added to the medium, B. coli in its growth breaks up the sugar into simpler products with the formation of gas. This is the kind of medium that is used to start the work of detecting the presence of B. coli in any material suspected of fecal contamination. In its production of gas, B. coli differs from its more pathogenic relatives forming the typhoid-dysentery group.

And this brings us to the few really harmful germs that occasionally dominate the intestinal inhabitants, if not always in numbers, in the detrimental effects they have on their host.

First there are those that are known technically as the food-poisoning bacteria. There are two very different groups of bacteria that cause food-poisoning, each acting in an entirely different way. One, called Bacillus botulinus, is a large spore-bearing bacillus from the soil that may be carried in vegetables and fruits, and because of its resistant spores may not be

killed, unless cooked for a long time at high temperature. It grows better when air is excluded (under anaërobic conditions), so if sufficient heat to kill the spores in the material has not been used in canning these products, this bacillus may grow very well later in the air-tight cans and produce a potent toxin that may quickly kill human beings. We will tell more about this bacillus in a later chapter when we introduce the other spore-bearing anaërobes.

The other group of food-poisoners belongs to the tribe we are describing here, one of those that may be truly called intestinal tribes. This group has been given the general name Salmonella after Salmon, an American who with Smith studied this group in the early days of its discovery. Its members are so closely related to the typhoid group that they are called the paratyphoid group, para meaning at the side of or near. They are near relatives. They are distinguished from the typhoid bacilli by forming gas in certain media, and more especially by their fermentation of the sugar rhamnose. They are different from the coli group in their inability to ferment the sugar lactose. They all look very much alike in form and staining.

A number of intermediate strains have been described. Indeed, there have been so many varieties described that in the naming of the species there has been more confusion than with any other group. The chief reason for this is that members of this group are, perhaps above all others, variable in certain traits. "Rough" and "smooth" varieties described in Chapter V were first discovered in this group. Mucoid and dry colonies and still other kinds of colonies may be found in subcultures from a single colony. Previous traits may even

be lost permanently. So the reports of each investigator concerning a stain were at first different in some respects from those of others. The classifiers are trying to correlate the various findings, and individual investigators are working hard to help clear up some of the apparent discrepancies.

That wonderful highly technical test, the absorption of agglutinins, spoken of in Chapter IV, is rendering great aid in throwing light on the confused relationships of this group of microbes.

Two members, called respectively paratyphoid A and B, are essentially human pathogens, producing usually a low-grade fever, something like typhoid fever. Krumwiede found quite a large percentage of normal human carriers of these types, so there must be considerable resistance to infection. In places where people from several scattered areas of country are herded together, as in camps during wartime, there is grave danger of outbreaks of infection from these types, so they are included with the typhoid bacillus in a vaccine recommended to all people who are going to places where they may be exposed to these bacilli.

They are all easily carried by milk, water, oysters and other foodstuffs, and so they may be considered food-poisoning bacteria, but the food-poisoners par excellence, those that have produced the unexpected, often fatal, outbursts of poisoning by food, are primarily pathogens of lower animals, and they belong in the majority of the outbursts reported to only one species of this group.

This most common one is the variety first discovered. It may produce inflammation of the bowels (enteritis) in any of the animals we use for food, and in other

animals, like rats, that might contaminate our food, and thus get into man. This was called by Gaertner, who first found it (1888), Bacillus enteritidis. It is now called Salmonella enteritidis.

The second frequent poisoner is also a common type causing infection among lower animals, but it is more often found in sheep, hence it is called the mutton type. Its scientific or species name?— This is a big bone of contention among classifying disputants. We may give it the first name de Nobelle, its discoverer, used, Aertycke, after the place where the first recorded outburst occurred, and call it Salmonella aertycke. It is ingested by man in eating insufficiently cooked infected meat, usually mutton or lamb.

The third type that has been implicated in food-poisoning outbreaks, though not so frequently as the others, is called Salmonella suipestifer. This organism was first found in hog cholera and for many years was thought to be the cause of this disease, until de Schweintz and his associates in 1905 showed that hog cholera was caused by a filterable virus and that this bacillus was merely a secondary invader. It may produce enteritis, however, in lower animals, and it has been found in some cases of human poisoning from food. Krumwiede found this form in a tapioca pudding that had been contaminated with infected pork handled by the one making the pudding.

Of course the strain found in the food eaten, the one found in the source that contaminated that food, and the one found in the people poisoned, must be identical. Some very clever detective work must often be done by bacteriologists to prove the case against a particular article of food. On the other hand, the clinical his-

tory of an outburst of food-poisoning may be so striking that it may be sufficient to implicate a food source of the microbe poisoner, even though the chain of evidence may not be complete enough for proof.

For example, in the very first outburst where bacteria were implicated, the classical one where Gaertner found his bacillus Salmonella enteritidis, a number of people came down with severe diarrhea and one died the next day. Gaertner found that the only food these people ate in common was beef from a cow that had been suffering from diarrhea when it was killed. The man that died so quickly was one that had eaten the most meat and eaten it raw! He began to have symptoms of gastro-intestinal disturbance two hours after eating. Gaertner found his bacillus in the spleen of this man—and in the meat of the cow—but even if he had not found this bacillus he would have been sure it was the cow's meat that had caused the poisoning.

The above incident illustrates several points in regard to this type of food-poisoning. In the first place, it shows that the amount eaten—that is, the number of bacteria taken in—determines the degree of infection. The man who died had eaten 800 grams. In the second place, it shows that infection is due to the growth of the bacteria through the tissues of man, rather than to any exotoxin produced, as in the case with the spore-bearing group of food-poisoners. This case also shows how quickly this type of organism may overwhelm the individual infected with massive amounts—symptoms in two hours and death in thirty-six. The case furthermore demonstrates how carefully our food should be inspected. In any sick animals bacteria may quickly pass to all parts of the body. Finally, the case indicates

that all suspicious foods, if they must be eaten, should be thoroughly cooked.

The quickness of the killing action of certain of these microbes on some people is still one of the many mysteries of the microbe world. Usually they haven't this power, and not all people are susceptible to them when taken in with food, so out of a group of people eating food contaminated with these virulent varieties there may be many who escape; but there is no way of showing who are susceptible or which varieties are dangerous. Hence, all of this group of bacteria must be considered potentially a source of danger. They may get into unprotected food in all sorts of ways.

Rodents may be a source of infection. Thus, Krumwiede investigated an epidemic of food-poisoning following the eating of a cornstarch-cream-filled cake where the filler was found to be contaminated with rat droppings. Both the shelf where the filler had stood uncovered overnight and the filler itself contained rat fecal masses!

The last group of bacteria classed as inhabitants of the intestinal canal of man, the most dangerous of all, though fortunately not frequent, is the typhoid-dysentery group, called Eberthella, after Eberth, who discovered its most important member, the typhoid bacillus.

The typhoid bacillus! What a microbe for producing tragic situations, the sources of which were long unsuspected! We mean those following in the wake of the human typhoid carriers. For the typhoid bacillus is one of the lingerers in a few human beings, fortunately in only about 2 per cent. of those that have had typhoid fever or come in contact with a source of infection.

It is bad enough to have typhoid fever, with its swollen and ulcerated Peyer's patches (patches of lymphoid tissue in intestines), its growth of the germ through other organs of the body, its irregular course possibly ending in death; but to become a carrier, a source of disease and death to others, is far worse.

One of the favorite resting places of the typhoid bacillus is the gall bladder. In some cases they even grow throughout the bile capillaries. The bile which dissolves the pneumococcus has no such effect on typhoid bacilli; on the contrary, it allows them to grow in it luxuriantly. Once they reach the gall bladder and the gall ducts through the capillary vessels, they may continue to grow there for an indefinite time, helping materially to produce the typhoid carrier.

This carrier may be quite normal otherwise. There is no way of telling that he is a carrier except by a highly technical and complex examination of his feces; or by the less certain and more harrowing way of following the trail of victims he leaves behind him. He may live unsuspected in a community, unless he is at all careless in his habits, then the dire happenings in his neighborhood point him out in this day of enlightenment as a suspect.

We all know the history of "Typhoid Mary." [1] But it cannot be too often repeated, because it illustrates so forcibly the great danger from carriers and the whole question of the control of this insidious situation.

She was a cook. What a comment on personal habits, particularly on the care that is taken of the cleaning of hands! Attention was first directed to her by Soper, who

[1] "Typhoid Mary," by George A. Soper, "Military Surgeon" for July, 1919.

gives a thrilling account of her detection and handling. Dr. Soper, who is one of our greatest detectors of the sources of epidemics, particularly of typhoid fever, was asked in 1906 by the owner of an estate on Long Island to investigate an outbreak of typhoid in his home.

Six people in a household of eleven had been attacked. No other case had occurred in that neighborhood. That ruled out general water, milk and other food supply. The individual water supply which the people firmly believed had been contaminated from the cesspool, privy vault or stable manure pit, one or all, was found on repeated examinations to give no evidence of fecal or other contamination.

The family had eaten a great quantity of soft clams obtained from an Indian woman living in a tent on the beach, and it was thought that she had dug the clams from polluted areas; but she also supplied other families who remained free from typhoid. That ruled out the clams as a source.

After thus going over minutely every possible source of outside contamination, including visits of members of the family away from home and visitors to the family before the outbreak, and excluding these, Soper concentrated on the immediate history of the household at this time.

At last he found the clue that led to the solving of the mystery.

The family had changed cooks about three weeks before the epidemic had broken out. The new cook was described as a tall, strong, healthy, intelligent SILENT Irishwoman who knew how to cook, and the family had been very sorry when she had said, about three weeks

after the outbreak, that she had to leave. As this cook had come just at the right time for starting the outbreak, and as the histories of all the other members of the family were found to be free from suspicion, Soper determined that this cook, the now notorious "Typhoid Mary," must be found. But she had completely disappeared.

Soper finally traced her to a family where she was acting as cook and where two cases of typhoid had broken out several weeks after she had arrived. He found that her reputation for silence had been well earned. She would say nothing about her past. When told she might be a dangerous typhoid carrier and that her history and the examinations of her discharges might clear up the situation, she indignantly refused both. It has never been learned whether she had really connected in any way the coincidence of typhoid cases with herself, but her leaving whenever the outbreaks occurred and her refusal to tell her history looked as if she may have had some idea of the connection. She insisted, on one or two occasions when she was pressed to answer, that no one had ever suspected her. The only incident in her life that she did tell about was one that happened in a family she cooked for in 1902. Soon after her arrival seven in this family of nine came down with typhoid. Mary herself was one of the two who escaped; and the father of the family, who had had typhoid early in his life, was the other.

Mary stayed and helped take care (?) of all the victims, and the father was so grateful to Mary that he made her a handsome present. Mary added, "That didn't look as if I was suspected, did it?"

It is true that until the time Soper took the situation

in hand none of the outbreaks following Mary had been attributed to her. They had all been investigated (?) and their sources decided, so no one was ready to incriminate Mary when Soper tried to find out about her, and he had great difficulty in tracing her history during those years. No facts pointing to her having had typhoid could be ascertained.

Soper, however, considered her suspicious enough to present her case to the New York City Health Department. The officials interviewed her, and when she still refused to have examinations made they compelled her to go to the detention hospital on March 19, 1907. There the necessary examinations were made. None of her examiners who knew her history were surprised when the laboratory experts reported that they found her feces TEEMING WITH TYPHOID BACILLI. Repeated examinations of her stools on an average of three times a week showed nearly always large numbers of typhoid bacilli.

Thus it was proved that "Typhoid Mary" was a living incubator for the typhoid germs, and that her soiled hands at toilet, insufficiently washed afterward—if washed at all—were the means of conveying the germs to her victims. In the outbreak that Dr. Soper studied so minutely it was considered that the infectious matter was carried by means of ice-cream containing cut up peaches which Mary had herself prepared. Here no heat sterilized the fruit handled by her.

Mary was transferred to Riverside Hospital on North Brother Island, where she was placed in a comfortable cottage and detained for three years. She was allowed to receive friends and was given work to do that did not endanger others. As this was one of the first

typhoid carriers detected in this country, the authorities scarcely knew what was the best thing to do with her. They suggested the removal of her gall bladder, which sometimes effects a cure, but she refused operation. Her friends and "Friends of Liberty" interested themselves in bringing legal action to have her released. But, fortunately for us, as you may know from her subsequent history, they did not succeed. The health authorities at the end of the three years consented to release her on her parole that she would not cook for others, or in any of the ways she had been taught to avoid in those three years be a danger to others; moreover, she was to report to the Health Department once a month. She promised.

She kept her word for a while, then she disappeared and was lost sight of for nearly five years, during which time she assumed various names, so it was very difficult later to trace her movements. Only two facts were found out.

She lived for a time with a friend who finally came down with typhoid. She was at a sanatorium in New Jersey where two cases of typhoid occurred. They had never had typhoid there before.

Then came the most dramatic demonstration in her history and the one that led to her rediscovery.

In one of the most capably managed maternity hospitals in the world an epidemic of typhoid broke out. Twenty-five nurses and other attendants came down with it. They had a fine cook known as Mrs. Brown, who was jokingly called "Typhoid Mary" when the epidemic began. This led some one to suspect her, and, unknown to her, one of the doctors from the Health Department who knew her was given an opportunity to

see her. It was Mary beyond doubt. Arrangements were made to capture her the next day. But by morning Mary had disappeared again. She was finally traced to a Long Island home, from which she had to be forcibly removed to her old cottage on North Brother Island, where she is to this day. She was considered too dangerous and irresponsible to be allowed to go free. Everything has been done for her comfort, and she seems on the whole now to be quite willing to stay there.

Mary was known to have caused ten outbreaks and fifty-one cases, among which was one death. She was probably responsible for many more outbreaks, considering her habits. As some one said, Mary was a fine cook, but she used indescribable spices.

The lessons to be learned from this first carrier to be found in America have been well summarized by Soper in his report already mentioned.

As a result of the facts learned from her life, the Health Department has passed a regulation that all food handlers shall be examined. Those found to be carriers must be registered and are not allowed to remain in a business that might endanger others. We in the New York City Health Department have registered nearly two hundred chronic carriers. Notwithstanding these controls, one of the food handler carriers recently went back to his pushcart business and dealt out ice-cream cones to school-children and others. As a consequence, a small epidemic of typhoid broke out in that neighborhood, which was quickly checked when the carrier was recognized.

Various means have been tried to rid carriers of the typhoid germs, but without much success. Of course, vaccination renders the majority of people immune for

a variable period. And as we said, this is advised for all those in contact with carriers, or those who are about to travel to regions that might be dangerous, or those otherwise to be exposed to infection.

The great sources of danger from wide-spread epidemic infection by the typhoid bacillus are milk, water and oysters. Danger from all of these is now minimized by special methods carried on by most health authorities.

The question of the use of typhoid vaccine was given a marked opportunity of being tested during the great World War. The results were unquestionably in its favor. At the age of the masses making up armies, from twenty to forty years, people are most susceptible to infection with the typhoid bacillus, and the vast majority of the troops developed no typhoid fever, a condition that did not obtain during the Spanish War, when the vaccine was used too late to be of service.

The only other important group of human intestinal pathogens is one composed of those that produce specific bacterial dysentery.

Many of the intestinal microbes were accused of causing specific dysentery in man before Shiga in Japan, in 1898, found the right one that causes the most severe cases. He called it Bacillus dysenteriæ. It is now known as Eberthella dysenteriæ. The only difference in appearance between it and the typhoid bacillus is that it is shorter and it is practically non-motile. But it acts differently on sugars, and it also gives a different clinical picture and causes the production of different antibodies.

This is the chief of the group, but there are many near relatives in different parts of the world. Park, who

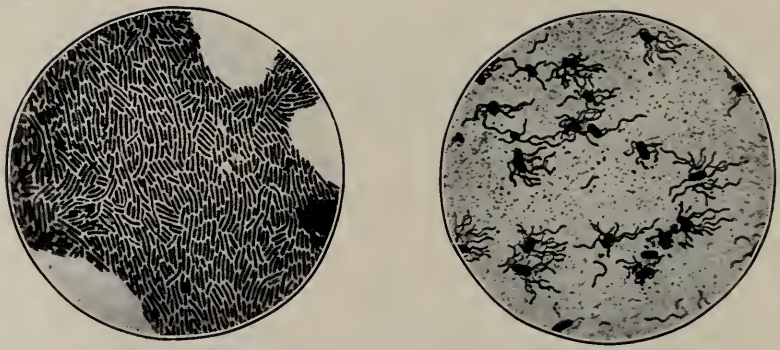
was the first to show that these relatives were different from the true dysentery bacilli, suggested that these be called Paradysentery bacilli, just as the near-typhoid relatives are called Paratyphoid bacilli.

These paradysentery forms are seldom met with in the United States, but the true dysentery bacillus is found even less frequently. The paras are not very virulent. But it is important to know that different types exist, so that we may learn how prevalent they are and be in a position to be able to trace any epidemic that may occur. In Japan these types may cause very severe epidemics.

Here, as in the identification of all other ultimate types of germs, the absorption of agglutinins test is used. In fact, it was by this test that Shiga, out of the mass of germs in the stools of his dysentery patients, was able to pick one that bore a relationship to the disease. Specific serum has been used with some success in the cure of dysentery cases, and a vaccine has been used as a preventive. Hygienic measures are carried out in the same way as for typhoid carriers.

Among the infrequent and transient inhabitants of the intestinal canal there is a small group of bacilli that are somewhat like the typhoid bacilli, but they do not act like them on sugar media. They do not break up any sugars. On the contrary, they make the medium more alkaline, hence they are called alkalignes. Among them is one that often reaches the intestines through goat's milk. It was found by Bruce very frequently in goat's milk in Malta and produced a fever called Malta fever. This occurred especially in soldiers. A number of people call these forms Brucella, after Bruce. Smith and others think this bacterium, which is so very short

Hunting for typhoid carriers, looking for colonies of typhoid bacilli in growths of dilutions, made from feces of case under inspection. When suspected colonies are found fishings are cultured for further study, and slide agglutinations are made to help complete identification



A B

A. Stained typhoid bacilli from 24-hour agar culture magnified 1000 diameters. B. Typhoid bacilli stained to show flagella

Making typhoid vaccine. Washing off the growth on nutrient agar in Blake bottle with normal salt solution and syphoning it into a collecting bottle

that it was first placed with the cocci, is a close relative of a similar form that causes abortion in mares, cows and swine. This variety is called Brucella abortus. They are both related to a little bacillus that is often found in dog distemper, though its pathologic relationship to that disease is not yet settled. The majority of the strains occurring in cow's milk are harmless in man, but a few cause a disease called undulent fever. Nearly a thousand cases have been discovered in the United States alone.

Of course any pathogenic organism taken with the food may start its infection in the alimentary canal, and there are certain varieties that may assume dangerous epidemic qualities whose chief site of action is the intestines. A notable one among this type is the cholera vibrio; but since this belongs to a different group of organisms, it will be considered in another chapter.

Theoretically, the disease-producing microbes found in the intestinal canal are easy to contror so far as transmission to others is concerned. All we need do is to disinfect the discharges of patients and of carriers.

Practically, the carrying out of these control measures is not so easy. In the first place, each person who has become infected with the typhoid bacillus, or with any other one of these germs, must be detected early in the disease.

The discharges may contain the bacilli even before the patient comes to the doctor. Then the carriers must be spotted. This detective work requires complex laboratory tests which take time and experts to carry out. Then we have to be sure that the attendants will follow our directions in the cleansing of their hands

and of everything that comes in contact with the patient's or carrier's discharges. We saw how difficult it was to regulate this in the case of "Typhoid Mary," and we are sorry to say that there are many more as careless as she.

In order that measures for the detection of carriers may be carried out, health departments employ a large number of milk inspectors, water inspectors and shellfish inspectors, and a large laboratory force to make the required examinations of the samples these inspectors bring to the laboratory.

## CHAPTER IX

## PASTEUR'S TRIBE

The "black death" bacillus—The ground squirrel or rabbit disease bacillus.

This tribe, called Pasteurella, was given Pasteur's name because Pasteur was one of the first to study the chief of the tribe and, while becoming acquainted with it, to make a great discovery concerning its behavior.

The chief, named Pasteurella avicida (avicida means a killer of birds), is a small Gram-negative bacillus that causes fowl cholera, or, as the disease is sometimes better called, fowl plague. Pasteur accidentally discovered when he injected into a fowl an old culture of this bacillus, in place of a fresh one, that the bird became sick; but instead of dying, as did the fowls that had a fresh culture, it recovered. Then, when Pasteur later injected it with a fresh culture, most unexpectedly it did not get sick at all.

Pasteur, thrilled with the thought that he had possibly discovered a new method of preventing fowl cholera similar to the method of Jenner for the prevention of smallpox, studied until he found out how he could attenuate the culture and regulate its dose so it would protect fowls absolutely from contracting the disease. As a result of these studies he made a bacterial vaccine which was the first bacterial vaccine ever used.

Each member of this tribe Pasteurella—and so far

there are only seven members known—produces a similar kind of sickness, but each one has a fondness for different kinds of animals. Some of them are scourges of our laboratory animals, and many are the researches they have interfered with by killing off test animals under observation. After the bacilli get into the body of their animal host they grow quickly and abundantly through the blood causing a septicemia. They also cause hemorrhages into the tissues, so they are often called the hemorrhagic septicemia group of bacteria. While the bacillus of fowl cholera, Pasteurella avicida, may be the classifier's chief of this tribe, it is not the chief offender in attacks on human beings. In fact, so far as we know, it never attacks humans. Only two of this tribe do this, and one of these lightly and seldom; but the other is truly a killer, a mighty power for evil to human beings, one of the worst among the big pathogenic microbes. For this is the microbe to which we have referred in our first chapter as causing in olden times those terrible wide-spread epidemics called the black death. These epidemics practically put a stop to all activities of man except his taking care (and what care!) of the sick, of the dying and of the dead; or, in his terror, his attempting to flee from his home and seek less infected regions, often without avail.

Think of the influence this bacillus has had on the world's history. Its behavior has occupied the attention of more historians, doctors, bacteriologists and purely literary writers than perhaps has that of any other microbe. We all know Defoe's famous description of the plague year of 1665. And other accounts of the ravages caused by this microbe are met constantly by readers of those periods of our world's history.

In the very earliest epidemics of plague recorded, over half of a population died of the disease. It is estimated that about twenty-five millions died of it during the fourteenth-century epidemic. This was truly a plague. The cause? The early people said it was the same as that of all other plagues, the judgment of God. Even if they had glimmerings of the part rats took in the spread of the disease, they thought that the rats were sent by God, and sacrifices or presents of golden rats and buboes (emerods) were made in the temple to the avenging God (see Bible—I Samuel, V and VI).

It was not until a comparatively recent epidemic occurring in China, in 1893, that the very small but powerful plague bacillus, as it is commonly called, or more formally Pasteurella pestis, was discovered. Two doctors, Yersin from France and Kitasato from Japan, working independently, discovered it at the same time. It is a short, comparatively thick Gram-negative bacillus, and, like all of this group, stains more intensely at each end: that is, it is bipolar in its powers to absorb stains. It does not have the brilliantly stained granules of the diphtheria bacillus, however. The plague bacillus may grow in more irregular forms than almost any other microbe, depending upon culture medium and age. These appearances all help in identifying the bacillus. Thus, if a little more salt than usual is added to the agar medium, the bacilli will show large swollen forms that are full of vacuoles or bubbles. Then in broth the bacilli grow in chains, looking almost like streptococci. In the tissues they grow usually as bipolar-staining, short bacilli. At the beginning of the disease there are enormous numbers of these bacilli

growing throughout the whole body. But they quickly swell up and dissolve in older lesions, so they may not then be demonstrated so easily. Usually cultures may easily be obtained from the inguinal or groin lymph glands, which are swollen, forming what are called buboes. Hence, the name bubonic plague is sometimes given to the disease. The fact that only this bacillus was found in all the tissues of people dying of plague, and was not found in any other disease, was strong evidence that it was the cause of the disease. But tragic evidence was soon to be added to this. Among the young investigators working in a laboratory in Vienna with a pure culture of the plague bacillus, one came down with a typical attack of the plague after a small wound infection. He quickly died. The bacillus was recovered in pure cultures from his swollen lymph nodes (buboes) and other tissues. Thus it was accidentally demonstrated that the smallest wound is sufficient to cause infection and that a pure culture of virulent bacilli may reproduce the disease. Since this first laboratory victim, in different laboratories of the world an occasional worker has infected himself while handling pure cultures of this bacillus. It may even be able to pass through the unbroken skin.

For this reason the plague bacillus is one of the few microbes that we do not allow to be given out of the laboratory to other workers, except to the Federal Public Health Service representatives and then under extreme precautions. This microbe may retain its vitality and virulence so long in the test tube, and it may infect people so easily, that we can never be sure it can be handled with safety except by the most trustworthy workers. Again, what a commentary on our personal

habits and on the insidious attacks of these minute creatures!

We have a strain of plague bacilli in our laboratory that Dr. Wilson kept under observation for years. He found that it had lived fully virulent in the test tube for over ten years without transfer, and this bacillus forms no spores. It is easily killed by heat and by the usual germicides. But under certain conditions, as the one just cited, it may hibernate for many years and still retain its death-giving powers.

It was several years after the discovery of the bacillus that the part played by rats and the rat flea was fully demonstrated. Thus, the deductions recorded so long before in the Bible were shown to be right ones.

The rat is now considered the chief host, the natural host of the plague bacillus, and the rat flea the chief carrier, or vector, transmitting the bacillus to other rats and to man. Bitten by the flea, man usually develops the bubonic form of the disease. In some people, however, the bacilli may cause pneumonia. This is a very virulent form, generally ending with death. As with other microbic diseases of the lungs, the germs may be conveyed to a new host by droplet infection. So people suffering from pneumonic plague are particularly dangerous to others, as well as in grave danger themselves.

The name pest bacillus (Pasteurella pestis) is well deserved. Even yet, with all our knowledge of its real hosts and its chief vectors and of its avenues of escape from these animals, as well as of ways of controlling them, there are still epidemics of plague in those countries where it is impracticable to apply this knowledge.

It is reported that 500,000 people in India annually

suffer from plague. These, of course, are a constant even if remote menace to the rest of the world. For this reason health authorities throughout the world, especially at the chief ports, are on the lookout for infected rats on vessels coming from infected countries.

While a number of rodents, perhaps all, are susceptible to the plague bacillus, only certain species of rats and marmots in various parts of the world, and the native ground squirrel in California, have been found actually to be infected with this bacillus.

Two varieties of the black rat have been found infected most frequently, but all animals found susceptible to laboratory infection are potentially dangerous; and, without care in protecting people against contact with such rodents and their fleas, risks of infection and even of epidemics may be run.

The rat flea seems to be very fond of its own host, which it scarcely ever leaves until the rat dies, or just before the rat dies. The fleas feed up to the last minute, and finally leave their dying host with their alimentary canals containing a full meal of the host's infected blood. Then they hop to another host, preferably the same kind of a rat. If that isn't in their neighborhood, any other rodent that may be present is chosen. They jump upon man only when no rodent is near. Before feeding on their new host the fleas regurgitate some of the infected blood at the site of the bite and bacilli are carried through the wound into the blood of the victim. If this victim is a rat it may only have a light attack and show no symptoms, or it may become quiet, creep into a corner and die, or it may develop chronic infection with few clinical symptoms. On autopsy the diagnosis in the majority of the infected rats may be made

quite easily with the trained naked eye. In the acute form all lymph nodes are swollen and may be hemorrhagic. The subcutaneous and muscle blood vessels are engorged. The liver and spleen are enlarged, and scattered through them are small areas of necroses or dead tissue.

In chronic rat plague there are foci of pus throughout the organs. The plague bacilli are recovered from these lesions by cultures or by the inoculation of a susceptible animal.

During and just after the World War we were so fearful of having our rats infected by the sick rats and their fleas that might be on vessels coming from infected ports, that the United States Federal Health Service obtained the coöperation of health departments at various ports to examine the local rats in order to find out the kind of rats in each neighborhood, the kind of fleas they harbor, and whether any rats gave evidence of being infected with the plague bacillus.

In New York City, which was one of the places chosen for the examinations, we examined thousands of rats and collected many more than thousands of fleas. We found no infected rats and none of the fleas that usually carry the plague bacilli.

In one of the southern ports a few infected rats were found. This warfare against rats and fleas is the chief measure necessary in the prevention of plague. Of course, in countries where human cases of plague still occur, the isolation of the patient is equally important. A vaccine has been found to give some protection, but the effects do not last for a long time, so its use is advised only in the face of an epidemic or for those people going to plague-infected countries.

The other bacillus of this tribe that is infectious for man and may occasionally cause his death is of great interest to humans for a number of reasons. In the first place, it was thought by McCoy, who discovered it in 1911, to be the cause merely of a plague-like disease of ground squirrels in California. McCoy and Chapin studied the organism and gave it the name Bacterium tularense, naming it after the country Tulare where the first infected squirrels were found. They found that the squirrel flea could transmit the disease. Rabbits have since been found to be quite frequently infected with this bacillus. The chief interest in this subject lies in the fact that when the habits of this bacillus were very minutely studied and a determined hunt was made for it to find out its geographic distribution, such as has been done by Francis of the United States Public Health Service, it was found to be not infrequently infecting man, and a number of doubtful cases of subacute human fevers were finally laid at its door.

Francis has given a clear description of all the clinical types of this infection. He has called the disease Tularemia, after the name given to the bacillus by McCoy. He found that it might be transmitted by several biting insects.

This bacillus resembles the plague bacillus in its ability to infect laboratory workers. It seems to get into the body of such workers very easily, usually in ways that cannot be traced. As met with in humans outside of the laboratory it usually occurs in marketmen, and transmission is effected through the wound that is accidentally made by the marketman as he skins and dresses rabbits for the market, infected ones among them; or it may occur in the housewife or cook as she dresses such

rabbits for the table; or a hunter dressing rabbits at the end of a day's hunt may infect himself. If any one handles an infected animal and then rubs his eye with his unwashed hand, he may become infected through the eye, developing the oculo-glandular type of the disease.

Man may also acquire the disease through the bite of the horse-fly or the wood-tick, both of which are carriers. A few have developed the disease from the bite of an animal, such as the coyote, just after the mouths of these animals have become infected from eating diseased rabbits. One case was of a mother who is believed to have contracted tularemia through the prick of her thumb, received while dressing the primary sore on her fly-bitten son.

Human cases have been reported in nearly all of the States in the United States.

The symptoms begin in an average of three days after the bite or wound. Then suddenly the infected one has headache, vomiting, chills and fever. When the regional glands are infected they are painful and begin to swell. Then at the site of the wound an inflamed papule appears, which soon breaks down, forming an ulcer with a punched out appearance. When this heals, scar tissue is formed. There may be nodules along the course of the lymphatics in man. In about half the cases the lymph glands suppurate.

Infection through the eyes may be very severe and occasionally fatal. There are certain cases in which the fever is the prominent symptom. These resemble typhoid fever. The diagnosis may be determined by the agglutination test. The disease runs a subacute course of several weeks, then gradually clears up.

Francis [1] sums up the important points relative to infection by this microbe as follows:

Laboratory workers handling this organism are almost sure to become infected in the long run.

Agglutinins are present in recovered cases. Abortus and melitensis cultures cross agglutinate.

The organism requires cystin for its best growth. It is very pleomorphic, it penetrates the unbroken skin and invades fixed tissue cells—the hepatic cells of a mouse and the intestinal epithelium of tick and bedbug. It is transmitted through the egg of the tick to the next generation of ticks. It has a great variety of insect and animal hosts.

Thorough cooking destroys the infection, thus rendering an infected rabbit harmless for food.

Laboratory workers engaged in performing necropsies of infected animals should wear rubber gloves and should observe all other precautions to avoid infection. Cooks, marketmen and hunters should wear rubber gloves in dressing rabbits.

The treatment is symptomatic. Rest in bed is the most important. Those who have had the most experience with the enlarged glands do not advise excision, or even incision, until a very evident soft, thin place appears in the skin overlying the glands. No preventive vaccine or curative serum has yet been perfected.

[1] "The Atlantic Med. Jour.," 1927, *30*, 337.

## CHAPTER X

## BLOOD-THIRSTY TRIBE

(Hemophileæ)

The intriguing influenza bacillus—The exciting whooping-cough bacillus.

Blood-thirsty (or blood-loving, according to the derivation of its scientific name) has a murderous sound when applied to any living beings, but as used to describe a prominent trait of these minutest of known living forms the name has not such a bad meaning as it might seem. The bacilli of this family are called blood-thirsty or blood-loving because they like blood in their culture medium, and imbibe it in their growth. Indeed, one form cannot grow in pure cultures outside of the body of its host unless it has some of the hemoglobin of the red blood cells, or a similar substance, in its food medium.

This form is the intriguing influenza bacillus or Hemophilus influenzæ, the chief of the tribe. We call it intriguing because it was long thought to be the cause of influenza, but now there are grave doubts as to its power to incite that disease. A few trained bacteriologists still believe in it.

When, during the big epidemic of 1889-93, Pfeiffer first saw immense numbers of this very minute bacillus in the sputum of those suffering from influenza he thought he had discovered the cause of the disease.

When he found it wouldn't grow in pure culture on any of the culture media that he tried he was sure he had a very mysterious unusual organism, and he continued his intensive study of it. He learned that it had no motility, that it stained faintly, decolorizing by Gram's method, and that it would grow along with some other bacteria from the sputum, but it would not grow alone. Finally, after many trials he found that it would grow in pure culture on artificial food when whole blood was added to its food. Then, after a very painstaking series of experiments, he proved that just one part of the blood was necessary for its growth, the hemoglobin. This fact, that it uses whole blood in its growth, is beautifully demonstrated when some of the bacilli are mixed up in a drop of blood and, after spreading the mixture over the surface of a nutrient agar plate, are allowed to grow at blood heat (37° C.) for twenty-four hours. If such a plate is taken out at this time and examined under the microscope with a low-power lens (magnified about 100 times), a brilliant picture is seen. On a red background of blood cells are scattered glistening white, rounded, slightly scalloped areas. These are the colony growth of influenza bacilli which have used up all of the blood cells in these areas.

To the naked eye these colonies look like minute drops of dew scattered through the blood.

Since the time Pfeiffer first discovered his bacillus the question of why it could only be made to grow in pure cultures when blood was added to its food has been the subject of many studies, during which some interesting facts have been brought to light.

Thus Davis, Avery and others found that there are two substances in the blood that have to be present

before the influenza bacilli will grow. Then they found that these same two substances were in a number of vegetables, and that good growths of the influenza bacillus may be obtained if one of these is added to its culture medium in appropriate mixtures instead of the blood.

Avery also found that certain kinds of soaps have an inhibiting effect on the pus-forming cocci but encourage the influenza bacilli to grow. This soap medium, called oleate blood agar, has proved to be a great help in isolating and identifying this bacillus.

It was also found that this bacillus would grow more luxuriantly if the blood that was added to the medium was heated. The blood is poured into the hot melted agar. The heat changes the agar to a brown color, making the medium look as if it were made of chocolate; hence we call this chocolate medium. We keep all of our stock strains of influenza bacilli on it.

When Pfeiffer first found his bacillus he was so sure it was the cause of influenza that he called the organism Bacillus influenzæ, and he tried to reproduce the disease in lower animals with it. But all the animals seemed to be quite indifferent to its influence. Then other microbe hunters begun to find the same kind of a bacillus in other diseases and even in normal throats.

What a disappointment this was to those who had great expectations of getting help from it in combating that scourge epidemic influenza! Pfeiffer and his followers cried, "There must be many carriers of the germ; that is the reason we find them so frequently in other conditions." "But," retorted the doubters, "if there are so many carriers, why do we not have cases of 'flu' more often? And why do we only once every

twenty-five years or so have a big epidemic of this disease?" As these questions remained unanswered, many came to the conclusion that this bacillus could not cause epidemic influenza. That it has some pathogenic power was conceded. They thought it might be a good second poisonous invader in mixed infections and that it might help to produce certain chronic inflammations. But as for this being the cause of the big pandemics that occasionally swept over the world, sometimes with high mortality—no, they objected, there must be a special virus that could do that.

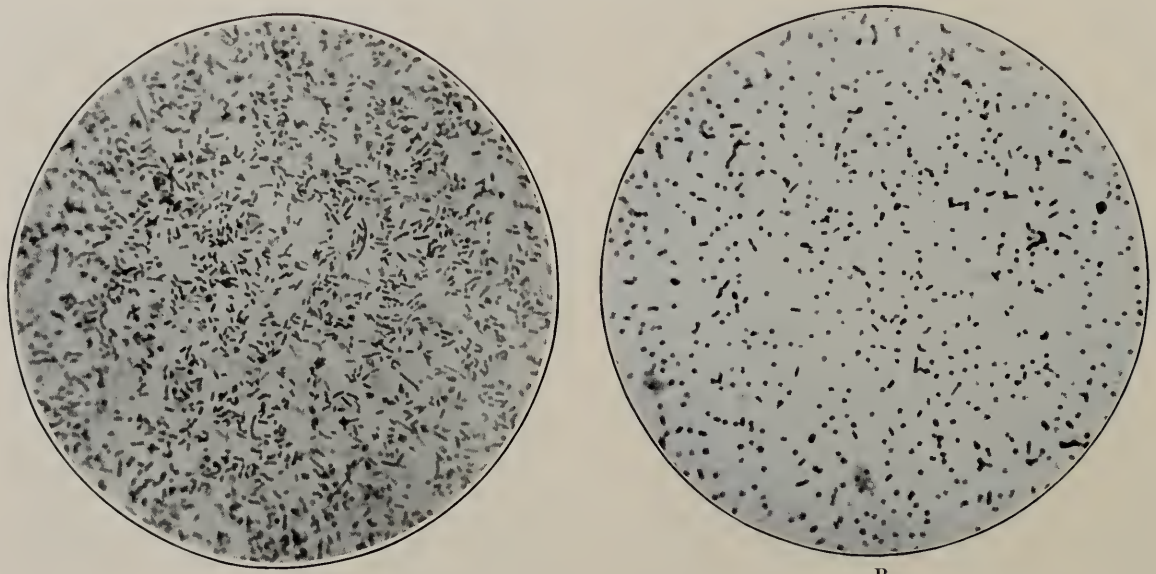
The opportunity to gain evidence as to its relationship to epidemics came with the last great pandemic during the World War in 1917 and 1918. This evidence was obtained by workers in various laboratories. We had plenty of opportunity at that time to study influenza under ideal conditions for obtaining an epidemic strain of this bacillus if it existed. Our hospital received many groups of sailors from different ships, each group having come down with influenza at the same time, and a big majority of these cases had great numbers of influenza bacilli in their sputum and nasopharynx. Besides studying these cases, we made a survey and gathered material from a number of camps while influenza was sweeping through them. We, alas! had many opportunities, too, for studying autopsy material.

We finally collected hundreds of strains of influenza bacilli and studied them from a number of angles, especially from that of their resemblances in serum tests.

We reasoned that if this bacillus caused the epidemic, then the strain from each case should give evidence of specific relationship to the majority of the other strains

Hunting for plague fleas on rats, in New York City Port

New York Health Department worker aiding Federal Health Service in its hunt for plague-infected rats



A B

The two best known members of the bloodthirsty tribe Hemophileæ. A. The influenza bacillus culture from chocolate agar; B. The whooping-cough bacillus culture from Bordet-Gengou medium. Both 24-hour growths. Both magnified 1000 diameters

we had gathered. Certainly when one infected individual transmitted the disease to a group, this should be so. We applied to each strain the serum test we have already mentioned several times, the one used for the identification of strains—namely, the absorption of agglutinins—and we found that by this test practically all of these strains were different. Therefore, we concluded, they could not have caused the epidemic, provided these bacilli retain their characteristics—their identity—on passage through human beings and after they had been isolated from humans. Many investigations were made to show that this bacillus might, in test tubes, quickly lose its virulence and other significant properties; but while some interesting observations were made, no decisive evidence was unearthed.

During this fatal pandemic many investigators searched rigorously for some cause other than the influenza bacillus, but no one found anything with specific significance.

Two workers at the Rockefeller Institute thought they had discovered it when they found in some cases of "flu" a very minute, filter-passing anaërobe which is now called by the imposing name Dialister pneumosintes. But alas! this must join the influenza bacillus in not coming up to expectations. No one else has been able to find it in influenza cases.

Further evidence against the influenza bacillus being the cause of the pandemic was given by the results from vaccinating with the bacillus. Such vaccinations seemed to afford no protection whatever.

So the cause of epidemic influenza remains as deep a mystery as ever, and we are still helpless in our efforts to combat it.

One interesting fact among the many brought out during these studies was observed by Dr. Povitzky, working in our laboratories. She found that nearly all the strains of influenza bacilli from a certain type of meningitis were related to each other. So we made a serum from these strains, and it has been used successfully in some cases of this type of meningitis which before this were always fatal.

There are several very near relatives of the influenza bacillus, none of them of importance to man except the one that has been accused of causing that very unbecoming disease, "pink-eye," or acute contagious conjunctivitis. This disease is now quite infrequent, so we know very little about the serologic relationships of the strains of influenza-like bacilli found in great numbers in the infected eye throughout the attack. The bacilli found in "pink-eye" have been called the Koch-Weeks bacilli because Koch and Weeks independently were the first to describe them.

There is another eye disease the cause of which is laid at the door of a member of this tribe. This is called angular conjunctivitis, because it begins in the angles of the eye. The bacillus supposed to cause it is a little bigger than the others of this tribe. It was discovered by two men, Morax and Axenfall. It is therefore usually called the Morax-Axenfall bacillus. The scientific name given to it is Hemophilus lacunatus.

Another member of the tribe is the cause of soft chancre in man. This, like all the tribe, will only grow in very special media. In this case blood serum is the best thing to add, and even then the bacillus makes only an extremely delicate growth.

Now we come to the last member of this tribe, and perhaps the most important from man's point of view, now that the influenza bacillus has been relegated to the background as an etiologic factor in epidemic disease.

This is the irritating whooping-cough bacillus, which is considered the cause of that dreaded disease of early childhood, pertussis, commonly known as whooping-cough. This bacillus was not discovered until Bordet, a famous Belgian doctor, assisted by Gengou, made a minute bacteriologic study of Bordet's two children as they were coming down with whooping-cough, and continued studying them during the whole course of the disease. They found early in the disease practically only one kind of a bacterium in the sputum that the children coughed up directly from their air passages during a characteristic paroxysm of coughing. This was a minute, Gram-negative bacillus that looked much like the influenza bacillus, only shorter and more regular in length. Indeed, several investigators have thought that the influenza bacillus was the cause of the whooping-cough.

Since the influenza bacillus often accompanies the pertussis bacillus, it is not to be wondered at that these germs were first mistaken for each other. But if they are once separated from each other in pure cultures the differences between them are marked. Bordet, at first, could not get his tiny bacillus to grow. Then when he added a large quantity of fresh blood to a potato broth agar and streaked over its surface a little of the sputum containing the bacillus, slowly after two or three days minute grayish, almost pin-point, colonies appeared along the streak. When a little of this growth was fixed

and stained, it was found to consist of innumerable minute bacilli like those seen in the direct stained film from the sputum.

The influenza bacilli do not grow as well on this medium as do the whooping-cough bacilli; moreover, they darken the medium as they grow, while the whooping-cough bacilli lighten it. The next and more marked difference between these two bacilli is that after two or three transplants on this medium the Bordet-Gengou bacillus, as it is sometimes called, will grow slowly but surely when transplanted to ordinary nutrient agar, while the influenza bacillus will not.

Then another difference Povitzky found out in our laboratory. If one adds just a little acid to the Bordet-Gengou food medium the whooping-cough bacillus will still grow well, while the influenza bacillus will not grow at all. This is a very good way to isolate the whooping-cough bacillus from the sputum when it contains many influenza bacilli and other bacteria that will not grow in an acid medium; and most will not. There are still other points of difference between these two germs, so now an expert bacteriologist has no difficulty in distinguishing between the two. The most important difference of all is that nearly all the whooping-cough bacilli obtained from different cases are alike serologically, while the influenza bacilli are not.

Bordet tried to find out whether any of the lower animals would get whooping-cough when he exposed them to infection with his bacillus, but none of them were at all disturbed by it. So he was not able to prove that his bacillus was the specific cause of whooping-cough by that method. Then he subjected the blood of his children to a number of tests which were at that

time quite new. He used the absorption of agglutinins test and the complement fixation test that we described in our fourth chapter. By these methods he found that as the children recovered from the disease their blood contained specific agglutinins and specific complement fixing substances. This was accepted as strong evidence that his little bacillus is truly the cause of whooping-cough.

If it is, said the questioners, then a vaccine prepared from it and injected into susceptible children should give them lasting immunity, since one attack of the disease does that.

So they proceeded to make a vaccine. The different methods of making this vaccine and the varying reports regarding its value both in curing and in preventing the disease would fill a large volume. The vaccine as made so far has probably some influence in preventing the disease, but it has very little, if any, in effecting a cure after the disease has once started.

## CHAPTER XI

## THE RESISTANT FAMILY

(Bacillaceæ)

Spore-bearers in the soil—Forms resisting canning—Anthrax bacilli—The lockjaw bacillus—War wound bacilli.

This is the great spore-bearing family, found chiefly in the soil. Its members form spores or seeds that are more resistant to deleterious influences than are the spores or cysts produced by members of other classes of microbes. They resist extreme heat or cold, prolonged drying and sunlight, the action of stains and of many chemicals.

They may indeed be called the resistant family.

Because of their power to resist destructive forces of nature they are found wide-spread throughout the world, in places where other varieties might not be able to exist. Wherever there is dust, there may be these spores.

They are the forms that so interfered with the clear understanding of our early microbe hunters on that mysterious subject, spontaneous generation, because they persisted in the boiled infusions of peas, nuts, meats and other substances used to demonstrate life. It took a Spallanzani, a Pasteur and a Cohn to prove that the irregular results following heating were due to the presence of resisting forms or spores.

But even they didn't realize how resistant to heat

some of these spores are. Certain varieties are reported to have withstood boiling in water 100° C. for nineteen hours. To be killed by heat in a much shorter time (one hour) they need to be superheated under pressure (310° C. or 600° F.).

It is the spores that we must be constantly on our guard against in sterilizing our culture media. Interrupted or fractional sterilization such as we described in Chapter II for allowing the spores to grow out into vegetative forms between sterilizations must sometimes be used.

More important still is the fact that these are the forms that resist the heat in the canning of our foods. Not only are the spores found on the vegetables and fruits we put up in our cans, but there are some especially resistant forms on sugar-canes. The canning industries and the sugar manufacturers have spent much time and money in investigating the powers of resistance of these forms. The question of their thermal death-point—that is, how much heat it takes to kill them—has been determined for all the species met with in these industries.

While the fact that they spoil our canned foods is important enough to occupy the attention of our canners, a far more serious quality of some of these forms occupies the attention of our physicians and public health officers.

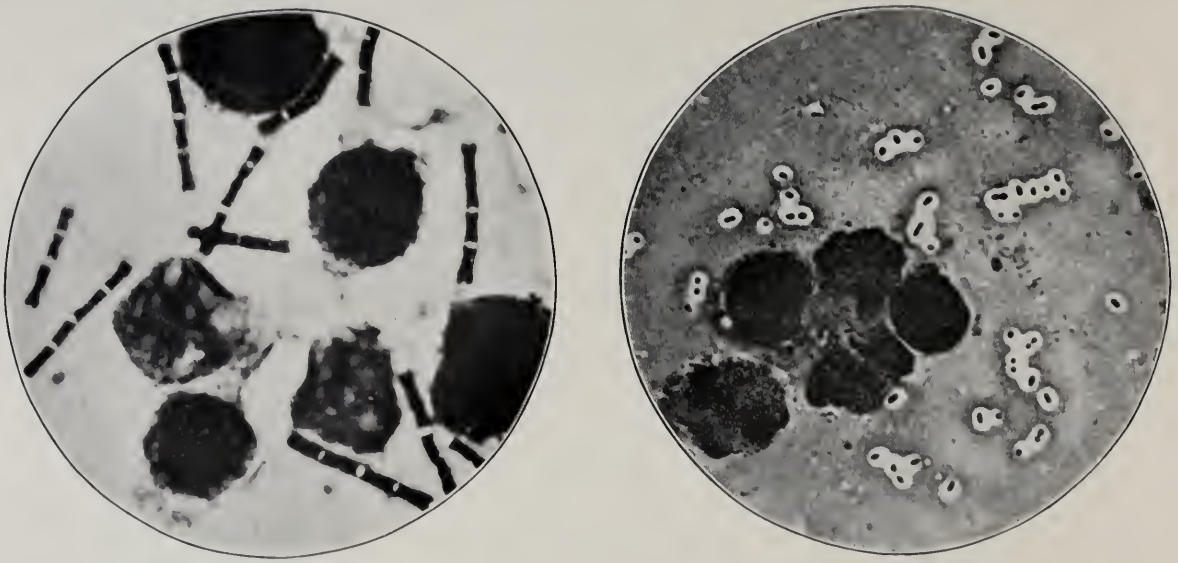
This is that they may injure our bodies as well as our foods and may do so to such an extent that death may result. We have in this family two of our most toxic destroyers of life and another one that destroys by its growth through the body of its victims. Then there are other members that produce less virulent

toxins and have less power to invade the body, but some of them may kill for all that. None of these forms produce definite epidemics; that is, they are not conveyed easily from person to person, or animal to animal. In other words, they are not contagious, but they or their poisons are infectious. They are usually picked up by the individual through wounds coming in contact with infected soil, or they may infect through their poisons, elaborate in infected canned goods or other preserved foods, which may be taken in through the mouth.

The resistant family is divided into two groups or genera chiefly on the basis of their behavior toward free oxygen. The first group is made up of forms that grow in the presence of oxygen—that is, they are aërobes. This group or genus is called bacillus, which means a little rod. The second group includes forms that cannot grow in the presence of free oxygen; that is, they are anaërobes. They are called clostridum, which means spindle, because many of the varieties assume this shape at certain stages of their growth.

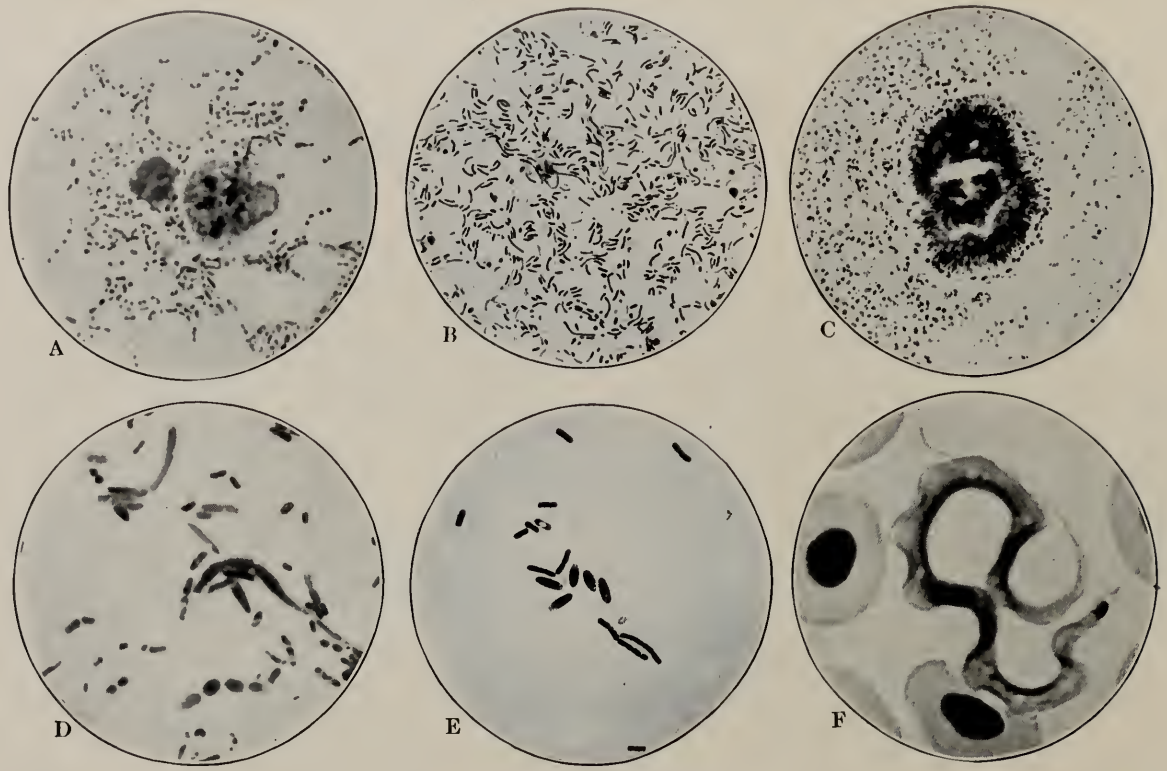
In the first group all of the varieties but one are either harmless saprophytes or they are indirectly helpful to man by the great activity they display in breaking down organic matter in the soil through their powerful enzymes.

The chief of this group, Bacillus subtilis, is of interest to beginners in bacteriology, because it is one of the first bacteria given him to study. It is so harmless, so big, so easily grown and stained; it produces such nice big spores so readily; it is so motile with so many big flagella. Moreover, it liquefies gelatine, peptonizes milk, produces acid in certain sugar media and hydrolizes starch; in short, it is a very useful and



A B

Microbes in tissues of test animals: A. Anthrax bacilli in liver of mouse showing "bamboo-rod" appearance. Enlarged 2000 diameters. B. Pneumococci in peritoneal fluid of mouse, showing capsules unstained. Enlarged about 1000 diameters (Fraenkel & Pfeiffer)




Some characteristics of certain important microbes: A. Diphtheria bacilli showing polar granules. (Fraenkel & Pfeiffer.) B. Cholera vibrios showing their comma forms. C. Rosette of the branching microbe Actinomyces growing in animal tissue (Schmorl). D. Swollen forms of plague bacilli grown on salt agar (Kolle & Wassermann). E. Clostridium (spindle) form of a spore-bearing anaërobe (Kolle & Wassermann). F. Fla-

convenient microbe to have around in a classroom so long as its spores don't find their way into places where they are not wanted. A close relative of this microbe, looking and acting much like it, only bigger, is also used for classroom demonstrations. It is called Bacillus megaterium. Both of these species look very much like the only harmful one in this group.

This enemy of man and beast is called Bacillus anthracis, from one of the names of the disease, anthrax, which means a burning coal. It was given this name because the animal affected usually has a burning fever and a darkened, almost coal-black blood.

It does not attack man primarily. Its favorite hosts are sheep and cattle. It also attacks horses and goats, but not dogs, cats or birds. While man is susceptible, he only contracts the disease through a wound or on prolonged contact with infected material.

This microbe is of great historic interest. It is the first bacterium that was shown to be the cause of a disease, the first microbe that was made to grow in pure culture outside of the body of its host and, after a number of culture generations, made to produce the disease when injected into a susceptible animal.

As usual, several investigators working independently helped to bring out all these new facts. Koch and Pasteur were the most active workers in this field. They knew practically nothing about making artificial culture media then. So Koch used the tears of an ox, and Pasteur used urine for cultivating this wonderful new microbe. They found that the rods are non-motile and that they grew out in chains of bacilli, end to end. The opposed ends are slightly concave, giving a bamboo-rod appearance to the chain. In gelatine, in which they

grew it later, it has been likened to a miniature inverted Christmas tree. In tissue it shows a capsule. It is Gram-positive.

One should read the life of Pasteur [1] to get a vivid impression of the enthusiasm and thoroughness displayed by him in these investigations into the cause and cure of the diseases that affect masses of men and beasts. At that time whole flocks of sheep were dying of anthrax, or splenic fever, as the disease was also called because the spleen became so big as the infection progressed.

Certain fields where the flocks fed were thought by the people to be accursed because nearly all the animals that went into them to be fed became sick and died. Pasteur soon showed that these fields were indeed accursed, but not in any mysterious supernatural way. He showed that the soil was full of anthrax spores deposited there by the dying and dead animals. Even if the dead animals were buried, as they usually were superficially in the fields, Pasteur showed that the earth worms feeding on them could take in their spores and bring them to the surface of the ground. He also showed that infection of fresh animals was hastened if the animals ate any irritating thing like prickly burrs.

Then Pasteur did a still more wonderful thing. He made a vaccine from these microbes that, when injected into susceptible animals, protected them against infection with the bacilli. That was another great day for Pasteur.

He had just made his chicken cholera vaccine. So this was the second successful bacterial vaccine made, both by Pasteur. The immunity following the anthrax

[1] By Vallery-Radot.

vaccine lasts about a year. All together, the measures recommended and carried out by Pasteur reduced anthrax materially in France. But owing to the imperfect working of the local health services in certain parts of the world, this disease still exists. The skins of animals that were infected with spores are used for various purposes. Wool-sorter's disease is anthrax contracted by workers handling infected pelts. The hairs of some of these pelts are used as bristles in shaving-brushes, and during and just after the World War, when many infected pelts were used, some of this material slipped through into our country and a number of people became infected with anthrax from such brushes. We examined the brushes and found anthrax spores in them. We recommended certain ways for sterilizing these brushes, which are now carried out.

While Pasteur was studying anthrax and devising ways for eradicating it, his attention was forced upon another bacillus by his detractors. They said they could produce a disease with anthrax bacilli in the blood and yet not get any culture from it. Pasteur worked over this situation. He was sure it wasn't anthrax; but blood containing it caused a septicemia in other animals, so he was equally certain that it was a poisonous microbe. Finally, he thought that the oxygen of the air might be interfering with its growth in artificial culture media. So he devised a way of excluding the oxygen, and, lo and behold! the thing grew beautifully along with other microbes. It was an anaërobe. The agar plate method of separating microbes and getting them in pure cultures had not come into use, so Pasteur only had the dilution method, which didn't always work; but he was finally able to

demonstrate clearly that this was an entirely different microbe from anthrax. Pasteur named this new organism Vibrion septique, because it causes a septic condition. It also causes swelling of the tissues, helped by the gas it produces. Koch found similar forms in animals, with intense local swelling or edema, that he called Bacillus edematis-maligni, but because he didn't get pure cultures we can't be sure as to its relationship to Pasteur's germ.

Pasteur called attention to the similarity of his new large anaërobic bacillus to the one he had described long before (1861) as causing butyric acid fermentation and which he had called Vibrion butyrique. This was the first spore-bearing bacillus that was shown not to be able to grow in the presence of atmospheric oxygen. This then is the recognized chief of the genus clostridium or spindle-shaped anaërobic bacilli.

Of course, this type of microbe, with its resistant spores and its frequent presence in the soil, was encountered often in the early days of bacteriology, and each discoverer, thinking he had found a new germ, described it under a different name. So now it has a list of twenty-six synonyms to indicate the interest it excited and the ignorance that existed in regard to its various characteristics.

Little wonder that in the beginning of bacteriologic time people's ideas were mixed in regard to the identity of an organism exhibiting such a protean activity.

In the first place, it is one of those energic fixers of atmospheric nitrogen that the Russian Winogradsky brought to light. Then it was used in producing butyric acid by fermenting sugar and putrid cheese. One of

the salts of this butyric acid has a very pleasant smell like pineapple. It is called essence of pineapple and it is used in making certain perfumes. It is also used in making artificial rum and other spirits.

Furthermore, this bacillus breaks up cellulose, coagulates milk, reduces nitrates, produces acid, gas and alcohol with many different sugars. Such is the chief of the spindle shapes.

It has a host of relatives in the soil and the intestines of certain animals and, through these sources, in many other places. Among them, as usual, there are some, including the Vibrion septique, that are dangerous to man. Two of these occupy a high place among the microbe enemies of man.

One is the dreaded lockjaw bacillus, the one whose spores lurk in garden earth, in the dust of streets, in unclean sand piles, around stables, on rusty nails and so on; in short, wherever manure in any form can reach. For these bacilli, the drumstick bacilli, so called because they produce rounded spores at the ends of the rods, make their home often in the intestinal canal of some domestic animals, particularly of horses. Not only do they grow in horses, thus making them carriers, but they may find their way into man's intestines and remain there as a potential danger.

The reason these carriers do not develop lockjaw is because they are immune to the tetanus toxin. They have antitoxin in their blood, and the reason the bacilli these people carry, and no doubt distribute around freely, do not cause lockjaw more frequently in other people is chiefly because the lockjaw bacilli can only produce their peculiar and very powerful toxin under special conditions, and because this toxin is poisonous

only for the nervous tissue, and is only fatal if it can get to the central nervous system over nerve tracks.

In other words, these bacilli, in order to prostrate their victim with that violently spasmodic disease called lockjaw or tetanus, must be able to infect through the right kind of a wound for allowing them to grow and manufacture their toxin.

Such a suitable wound must be dirty and ragged to allow other bacteria to be introduced at the same time so they may grow and use up the oxygen that is so harmful to the tetanus bacilli and others of this group. Among these associated bacteria, furthermore, must be none that produce certain acids in their growth, because these acids may interfere with the production of the tetanus toxin. When these fastidious tetanus bacilli find just the right conditions, they grow quietly but steadfastly in the wound, causing no apparent disturbance there but manufacturing slowly and surely that deadly toxin which is absorbed by the motor nerves and carried to the brain, where it accomplishes its distinctive intoxication causing those violent rigid spasms and usually the death of the victim in a short time.

So beware of dirty ragged wounds! These are the wounds that occur so frequently in wars. And in the old Fourth of July days, how many cases of tetanus followed the wounds of the shot-up children! Not only are wounds that are caused by explosives more ragged and more dirty than most other wounds, but gunpowder often contains the spores of tetanus bacilli carried in on the cotton gun-wads.

In the old days, too, of septic midwifery, there were a number of cases of tetanus following childbirth, and

these may be common yet in those parts of the world that are not reached by our laws of hygiene.

Owing to the ubiquity of the spores, special tests must be made to preclude their occurrence in any of our biologic products or in any of our surgical dressings.

Fortunately for the careless or ignorant, these bacilli, like the diphtheria bacilli, have been shown by man to be able with his help and that of a susceptible animal to provide their own prevention.

That powerful toxin of theirs, when injected in carefully graded, gradually increasing doses into a susceptible animal like the horse, will cause an equally powerful antitoxin to be manufactured.

This antitoxin, when injected into any one immediately after receiving a wound that might be infected with the tetanus bacilli or its spores, is almost a 100 per cent. preventive of tetanus. The injection must be repeated at the end of ten days, as the antitoxin has disappeared by this time and with it the immunity.

For many years, now, the health departments supplying antitoxins and other biologic products to the citizens send to the supply stations, just before the Fourth of July, enough tetanus antitoxin to have on hand for injecting in cases of firearm wounds. Since the development of the "Sane Fourth," not so much of this serum has been used, but plenty of it is still kept conveniently near in case of need.

At the beginning of the World War, when the supply of the tetanus antitoxin serum was low, many of the wounded died of lockjaw. When the antitoxin was rushed from all possible producing plants to the front,

and began to be used freely and often, there were practically no more deaths from tetanus.

This antitoxin is used very frequently in veterinary practice, particularly in the treatment of horses that are so susceptible to the toxin.

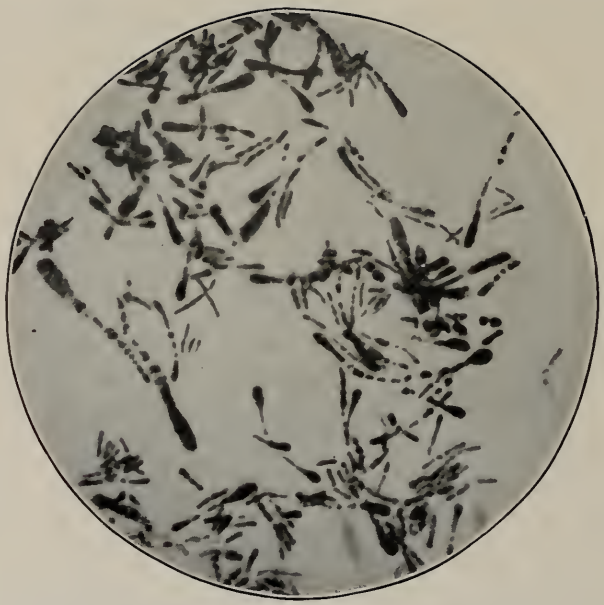
In our own work with animals we inject all horses with a preventive dose of this serum before we start them on their course of injections for the stimulations of their various antibodies.

Prevention, then, is the great useful property of this serum. A vaccine can be prepared from the toxin by adding a small percentage of formalin and allowing it to stand for a month at body temperature. Its injection causes no bad effects; on the contrary, it causes an active immunity to develop. If we ever have a great war again we will probably vaccinate all the troops, just as we now do against smallpox and typhoid fever.

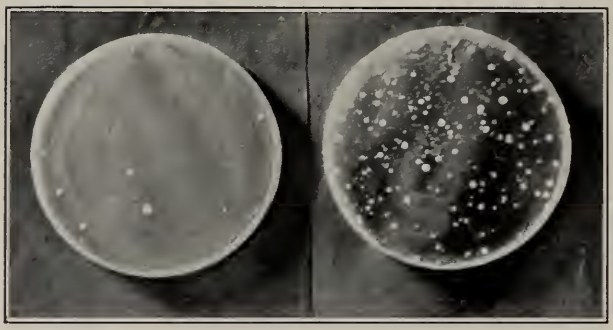
Cure is another matter. After a person once shows symptoms of tetanus his cure is doubtful. It used to be in greater doubt than it is now since we have learned that if the serum is injected into the spinal canal it reaches and neutralizes the toxin much more effectively. So now only one quarter of these victims of tetanus die, while before the intraspinous method of injecting the serum came into use fully half of these became rigid in death.

The other dangerous enemy of man in this group is the one that causes those spectacular cases of food-poisoning known as botulism, so called because the first cases described in the latter part of the eighteenth century followed the eating of certain sausages. Many cases called ptomaine poisoning were no doubt caused by this bacillus.

Technic of making culture from diphtheria throat. The cotton swab which has been rubbed over the infecte tonsils of the patient, and then over the surface of the coagulated blood serum media in the upper tube, is being returned to its glass tube. Both tubes will have the cotton returned to them and will be sent to the laboratory. The culture tube for incubation and examination



Diphtheria bacilli showing large club-shaped forms. Grown on nutrient agar for 48 hours. Magnified 2000 diameters



Comparative colony growths from clean and dirty milk, grown in nutrient agar in Petri dishes. Reduced to one third diameter

After the bacillus (Clostridium botulinum) was described by Van Ermengen in 1896, and its characteristics studied, it was found to be wide-spread in nature. It has been isolated from fruits, hay, vegetables, from the feces of various animals, and from garden soil in different parts of the world.

From these sources it gets into our food, and if its spores can develop there, its highly potent toxin may be formed. It grows without air, so if the spores have not been killed, our air-tight canned foods may be ideal places for it to grow. It doesn't produce any smell in its growth, so we cannot detect its presence by means of our noses as we can other putrefactive microbes. It has been found in canned olives, beans, asparagus, corn, spinach, pears, apricots and beets, besides being traced to cheese and to sausage and other preserved meats and fish. This is one of the poisons that may not be destroyed in the stomach.

The housewife's chief protection is in boiling any foods that have the slightest suspicion of being infected with this bacillus. The poison is killed by fifteen minutes' boiling. Known infected food should be barred. If thrown out to animals, they too may be poisoned. Chickens may get limber-neck. Hogs, horses, cattle and goats have been shown to be susceptible. People and animals infected show more or less rapid symptoms of paralysis.

An antitoxin has been produced which prevents the action of the toxin in experimental animals, but it does not have much influence in curing a case in man or beast showing definite symptoms of botulinus poisoning. The remedy is applied too late.

Another member of these poisonous soil spore-bearers

growing only without air is one that so far has not been found in man, but it produces a disease called quarter evil or black leg or symptomatic anthrax in sheep, cattle and goats. Other domestic animals and man seem to be naturally immune. A vaccine was prepared by Arloing, after the manner of Pasteur's vaccine against anthrax, which with modifications is still successfully used. The bacillus is called Clostridium chauvei.

The other pathogenic members of this genus are grouped under the name of gas gangrene bacilli. They include the one first found by Pasteur and called Vibrio septique, the one found by Welch known as B. welchii and many others.

Studies of them during wars have shown that they are the chief cause of the gaseous gangrene following those dirty lacerated wounds so frequent in war, so rare in peace. These have been called the war wound bacilli.

Volumes have been written of the investigations of this group and still there is much confusion in regard to their relationships. Many names have been used for the same organism, and many have been the discussions and refutations in regard to the results published.

Practically it has been learned from all this study that such wounds usually contain several varieties of this kind of microbe, some pathogenic and others merely harmless companions. It has been shown that probably only six varieties can take part in the process of gas gangrene. Of course, in these wounds there may be dangerous streptococci and other microbes as well. All of these have to be taken into consideration in the treatment of badly contused, dirty wounds.

A number of the anaërobic bacilli found in these

wounds during the World War were shown to be able to produce a toxin from which antitoxins were prepared, but we know little of the practical application of these antitoxins, for by the time they were ready to be tested in human beings the war was over. Theoretically a polyvalent antitoxin made from the kinds of bacilli most frequently found, including the tetanus antitoxin, should be injected as a prophylactic measure in cases where wounds of this kind cannot be adequately treated locally.

## CHAPTER XII

### THE CLUB-SHAPED GROUP

(Part of the family Mycobacteriaceæ)

The diphtheria bacillus clan and relatives.

This is a very large and important group, large enough and important enough and varied enough to be considered a family, but it is still only classed under the term (genus) Corynebacterium, meaning club-shaped bacterium. The bacilli in different ramifications of the group, in the different species of the genus, differ in shape and appearance. Many are club-ended, others more pointed. Some members have special staining and other characteristics. These differences distinguish one strain from another.

The most important member, the diphtheria bacillus, whose formal name is Corynebacterium diphtheriæ, makes its habitat almost entirely in the human race. Occasionally it is transmitted from man to an animal. Thus in a few cases it has been found in pustules on the teats of cows, and in one case we found it in the throat of a cat. There has never been a determined hunt made for it in lower animals, so these may be carriers more frequently than is suspected. It grows fairly well in cultures on nutrient jellies and in broth when kept at blood temperature, but especially well on blood serum media.

The diphtheria bacillus has a long record as an

enemy of the human race. It can be traced back over 2000 years in the histories of cases of undoubted diphtheria. It used to occur in only occasional outbreaks, but as the population became denser and travel more frequent, diphtheria began to be continuously present in the cities, being much worse during some years than others. People living in the colder climate grew to fear it, for it became the most dreaded disease of childhood, and it also very frequently attacked grown people.

The diphtheria bacilli have the peculiar function of producing a strong toxin which is found not in the germ but in the matter in which it grows. Grown in suitable beef bouillon at blood heat for some days, Roux, who is now director of the Pasteur Institute in Paris, found that it made the bouillon toxic. To prove this, Roux separated the broth from the bacilli by passing it through a porcelain filter which keeps the bacilli back. The filtrate contained this very powerful toxin, a few drops of which sufficed to kill a horse if injected into its tissues. This, as stated in Chapter III, we call an exotoxin, since it is excreted into the fluid in which it is growing and is not contained in the bodies of the bacilli.

A case of diphtheria develops when one or more of the bacilli reach a susceptible throat, and having lodged on the mucous membrane the bacilli multiply and begin to form their toxin. The toxin acts on the cells of the mucous membrane, so causing injury and making an even better soil for the growth of the bacilli. The germs develop more toxin and this causes more injury. Serum is exuded, epithelial cells become necrotic, bacteria increase, and thus we have inflammation and the development of a gray pseudo-membrane. Some of the toxin

is absorbed into the deeper adjacent tissues and they become swollen. If the case is a serious one, part of the toxin passes beyond the tissues involved to the lymph channels and through them to the blood. Escaping from the blood capillaries, it passes to all parts of the body. Then it acts on different parts of the muscular and nervous systems, resulting in early paralysis of the heart or of the respiratory center, or later of some of the voluntary muscles. If the disease affects the laryngeal mucous membrane, the inflammatory reaction results in swelling which causes the loss of voice, then difficulty in breathing and finally suffocation, unless relief is given by inserting a tube into the larynx.

One of the most important peculiarities of the diphtheria bacillus is that it is frequently found in the apparently normal throat, and not only lingers there but multiplies. It behaves somewhat like the typhoid bacillus in the gall bladder of a typhoid carrier. It thus remains a parasite in a healthy person and may be passed to others who are not immune. This is one of the ways in which cases of diphtheria arise. Here is a striking example.

Some years ago a nurse attended a group of cases of diphtheria occurring at a summer resort. On their recovery she went to take care of a child in a family living several hundred miles away. Shortly afterward the only child of the family developed severe laryngeal diphtheria. One of us attended the case and found bacilli of the same characteristics in the nurse and the child. This taught us that a nurse who cares for a case of diphtheria should have repeated cultures made from her throat and not be allowed to go to other children until these cultures show no diphtheria bacilli.

In 1884 the human race first began to make the acquaintance of the diphtheria bacillus. A German professor named Klebs noticed that when a bit of the pseudo-membrane from a characteristic case was smeared over a glass slide, stained with methyl blue and placed under the microscope, it showed a large number of the club-shaped bacilli and bacilli with more intensely stained ends that we have just described. He found, on the other hand, that when he smeared the exudate from cases which were probably not diphtheria, such as cases of inflamed throats in scarlet fever, he did not see similar bacilli.

The next year another German professor, named Loeffler, not only confirmed Klebs's work but also isolated the germ, grew it in pure culture and produced diphtheria in rabbits. Then, as already stated, Roux discovered that the diphtheria toxin was an extracellular one and that this toxin was the cause of the throat inflammation, the exudate, the heart failure and the paralysis of the voluntary muscles. These discoveries led to practical applications which have revolutionized our methods of treatment and prevention.

In 1892 one of the writers thought that we could use our knowledge of the appearance and cultural traits of the diphtheria bacillus to separate cases of throat inflammation due to the dangerous diphtheria bacillus from suspected cases due to the less dangerous streptococci. This thought led to the development of the first public health bacteriologic laboratory.

In the spring of 1893 the Health Department undertook to make cultures from all suspicious cases and to isolate only those in whom these bacilli were found. The cultures were also made to clear cases after con-

valescence. On the twelfth day a swab was gently rubbed in the crypts of the tonsils and against the nasal mucous membranes and then stroked over the surface of coagulated blood serum held in a glass tube. The inoculated culture was then taken to a laboratory and placed in an incubator at about 97° or 98° F. for ten to twelve hours. At this time any diphtheria bacilli present would have increased more rapidly on this especially chosen medium than the other throat bacteria. A little of the bacterial growth is then removed from the tube and spread on a slide, stained and examined under the high magnification lens. If any diphtheria-like bacilli are present they will be discovered, and the person either has diphtheria or is a carrier of diphtheria or diphtheria-like bacilli. If there are no diphtheria bacilli in the culture, the case is probably not one of diphtheria. The reader notices that these statements are not absolutely positive.

It is now known that in the diphtheria group there are certain members which have no power to make diphtheria poison or toxin. These are in every other respect members of the dreaded diphtheria clan. They can only be detected by testing them on guinea-pigs, animals that are very susceptible to the toxic diphtheria bacilli. If a case does not look like diphtheria, we suspect the bacilli may be of the non-poisonous type. These are called loosely non-virulent diphtheria bacilli.

There are also other bacilli found in the throat of human beings that resemble diphtheria bacilli in many respects, but besides making no toxin they make different ferments for sugars and are shorter and more regular in form. These are called the pseudo-diphtheria bacilli. Their registered name is Corynebacterium

pseudo-diphthericum. They used to be called Bacillus hoffmanni, after one of their early introducers. They are of no known importance except that they may at first confuse a bacteriologist who is not fully acquainted with this class and its relations. Other unimportant relatives are numerous, so a bacteriologist has to be very well acquainted with them all in order to rule them out as being dangerous if they appear in the culture he is examining from a case of suspected diphtheria.

Thus, one of the varieties that looks under the microscope very like a true diphtheria produces a yellow color in its growth. This was found for the first time by some of our Health Department workers long ago in a sample of cheese that was sent in for examination because it was suspected of causing some cases of diphtheria. We soon showed that this bacillus, now called Corynebacterium flavidum, is a frequent habitant of milk and all of its products and that it is entirely harmless for humans.

Then there is that very regularly barred bacillus that is found so often in the normal linings of the eye and nose. It goes by the name Corynebacterium xerosis and it too is quite harmless.

There is another variety that may not be so harmless which may be mistaken for the diphtheria bacillus by the uninitiated. This is the one that is considered to be implicated in that bad ulcer of the throat known as Vincent's angina. It is an anaërobe and grows so differently otherwise from the diphtheria bacillus that it is considered another genus. It goes by the name Fusiformis dentium.

If no diphtheria bacilli are present in the throat, the case is not one of diphtheria and cannot produce diph-

theria. Unfortunately, the absence of the bacilli in a culture does not absolutely prove these are not in the throat, since it is possible that the swab did not reach the exudate or that the culture medium was too dry or improperly made, so that no growth could develop. The absence of the bacilli in the culture is considered of sufficient importance to free suspected cases from isolation, but not to stop the use of antitoxin in any person who seems to have diphtheria.

The next step in the campaign of the human race against the diphtheria bacillus was the discovery of antitoxin. Behring, a German bacteriologist, in 1892 began work upon the possibility of protecting guinea-pigs against diphtheria, with the hope that if he succeeded he might obtain something useful for combating the disease in man. Nearly all the bacteriologists at that time were trying to find protective substances against the different poison-forming bacteria. He utilized a weak solution of iodine to attenuate the living diphtheria germs and injected these in increasing quantities into guinea-pigs. After about six weeks to two months he found that the guinea-pigs had become quite resistant. In trying to discover the cause, he found one day that the blood of the guinea-pig which had been immunized would neutralize the poison of the diphtheria bacillus which we have already stated was discovered by Roux. Later he tried giving the toxin itself and discovered that it stimulated the production of an antitoxin in the guinea-pigs. Larger animals were then used and in 1894 he and Roux gave a paper describing the beneficial effects of diphtheria antitoxin when given to those sick with diphtheria. They also believed that it would be of value in preventing diphtheria.

In the fall of 1894 we had an opportunity of testing these facts ourselves. In a children's institution at Mount Vernon a severe attack of diphtheria developed. Cultures from the children's throats were repeatedly made with the endeavor to eliminate all those who had diphtheria bacilli, but in spite of this the disease spread. At this time Dr. Hermann M. Biggs came from Europe with a supply of the diphtheria antitoxin and we persuaded him to let us use half of his supply in stamping out diphtheria. We gave the next morning a small injection of antitoxin to each of the children and the disease stopped. Twelve days later a very mild case developed. We again immunized all the children in the building, and from that time no more diphtheria developed. This taught us two important things—that diphtheria antitoxin prevents diphtheria but also that the prevention is only for a short time. This rapid decrease in antitoxin in the body of the human cases, we learned, was due to the fact that the diphtheria antitoxin was produced in an animal of a different species from a human being. We obtain our antitoxins chiefly from horses. The horse receives every two or three days, under the skin, an injection of diphtheria toxin. The amounts are at first small until after two or three months, when each injection contains enough to kill a thousand horses, but the horses show no visible reaction to the injections of the toxin. Because of the antitoxin then in the horse, it has no effect. Now it has been learned that when you inject one species of animal with the blood of another animal, the foreign blood is eliminated after a few days.

A little later, we did this very pretty experiment. Having in the laboratory some antitoxin made in

guinea-pigs and some made in horses, we injected each of a bunch of guinea-pigs with a little of the antitoxin produced in guinea-pigs and we injected another bunch with a little antitoxin produced in horses. The amount used was what we call ten units. A unit is approximately the amount of antitoxin which neutralizes a hundred fatal doses of toxin for a small guinea-pig. At the end of two weeks we took a guinea-pig from each of the two bunches. The guinea-pig that had received the antitoxin made in its own species showed no harmful effects from the toxin; the guinea-pig that received the antitoxin made in the horse developed an ulcer where it was injected. The next week another guinea-pig was taken from each bunch and injected with two fatal doses of toxin. Again the guinea-pig which had received guinea-pig antitoxin withstood the injection without the least damage, while the other died. Then for seven months we occasionally took guinea-pigs from the bunch receiving the guinea-pig antitoxin, and it was not until the end of that time that they began to be poisoned.

Encouraged by this experience, the Health Department of New York City, through its medical inspectors, treated yearly more than 15,000 children who had been exposed to diphtheria in their families. Of these 15,000 only twenty-five showed any symptoms of diphtheria, and none of these was severe.

Antitoxin used in the treatment of diphtheria is as much a preventive as when used to prevent diphtheria. Its only action is to meet the toxin, combine with it and neutralize it. A very good analogy is the effect of water on a fire. Just after a building catches fire a very little water will put the fire out; a little later

the extent of the fire will have increased and a part of the building will be ruined. Still it may be repaired. If the fire is allowed to burn longer, the building is destroyed and, even though the water extinguishes the fire utterly, the wrecked building cannot be made fit to live in again. Antitoxin, therefore, must be used in diphtheria at the earliest possible moment and in a large enough amount to be sure of neutralizing all the poison. If used early enough, the disease will surely terminate in recovery. If used later, the outcome is doubtful, and there comes a time when it may have no effect.

With the use of diphtheria cultures to prevent the dissemination of the bacilli by convalescent carriers and by healthy persons we were able to reduce in some measure the number of diphtheria cases. With the use of diphtheria antitoxin to prevent diphtheria in those already infected or to cure diphtheria in those having the disease, a greater reduction in the number of diphtheria cases and a much greater one in the number of deaths occurred. In New York City, for instance, the deaths were reduced to one seventh of what they formerly were between the years 1895 and 1915. We found, however, that many cases were not diagnosed as having diphtheria until the poisoning had progressed so far that no antitoxin could remedy it, and that many undetected cases carrying diphtheria bacilli scattered the disease. Not knowing who these persons were, it was impossible to immunize those whom they could infect. It has already been stated that the horse-made antitoxin would remain in the human body for only about two weeks; it was therefore clearly impossible to give every child a dose sufficiently often to keep it

immune. We found, then, that in spite of the great advantages brought by antitoxin, there were in 1914 in New York City, for instance, still about 15,000 cases and some 1400 deaths. It became obvious that something further must be done if we were to eradicate diphtheria.

We knew that through a substance called toxin-antitoxin or still another substance called toxoid we could immunize animals. The first substance was the toxin modified by antitoxin so that while it would not be irritating it would still produce immunity when injected into the children. We knew that the toxin acted upon by formalin, called toxoid, would be changed in a similar way. We knew that animals receiving these injections would remain immune for about two and a half years. We spoke often of using either one of these mixtures in immunizing children. There were great practical difficulties, however. We knew that about one half of the children and four fifths of the adults were immune in the large cities. These had become so partly at least through becoming carriers of more or less attenuated bacilli. How were we to tell which children needed to be made immune and which children were already immune? The only way available to decide this was to take a sample of blood from each child and test it for antitoxin on guinea-pigs. If the children turned out not to be immune and we tried to immunize them, we could only estimate our success by again bleeding them and again testing. Then finally to discover how long they would remain immune we would have to bleed them from time to time and test out their blood on guinea-pigs to estimate our success. This was

not a practical method of doing the immunization on a large scale and of studying the results.

A Viennese physician who now lives in this country, Dr. Schick, thought of slightly modifying a test we had long used in animals and applying it in children. In this test we injected a definite amount of antitoxin into the body of a guinea-pig. Then we injected into the skin of this pig small amounts of different cultures which we suspected were diphtheria bacilli. In another pig we injected the same cultures but no antitoxin. If any of these cultures were diphtheria bacilli they would produce a little area of inflammation in the skin of the pig that had received no antitoxin, but no reaction in the skin of the pig that had received antitoxin. A modification of this test was to inject mixtures of toxin with different amounts of antitoxin into the skin. Schick reasoned that if a child had any antitoxin in its blood it would have it in its tissue fluids; and the idea occurred to him that if he injected a very tiny amount of diphtheria toxin into the skin and the person was immune, there would be enough antitoxin in the plasma of the skin to neutralize it so that no appearance of poisoning would show itself, while if no antitoxin existed the toxin would act on the skin cells and a slightly raised reddened area would appear. Fortunately, this idea proved to be correct and the Schick test became available. Immediately we saw that in this test we had an easy method of differentiating the immune from the non-immune and an easy method of determining how soon those who were injected became immune and how long that immunity lasts.

In order to test this out thoroughly we approached

the authorities in charge of a number of children's institutions, telling them that we were sure that the test would do no harm and that the immunizing injections would benefit the children. We were able to test more than 10,000 small children. We found the Schick test in differentiating the immune from the non-immune to be very reliable. We found that in about four weeks after receiving the injections some of the children became immune and that most of them became immune within three months. About 10 per cent. of these young children required a second series of injections.

We then began to test from year to year to see how long the immunity persisted. We have continued this testing for ten years and find that in about 80 per cent. the immunity has lasted. Those that relapse can very readily be reimmunized by a second series of injections.

In order to determine fully the value of toxin-antitoxin or toxoid injections in practically immunizing the child population, we and others carefully watched the results. We tabulated 200,000 school-children, 100,000 of them being untreated and 100,000 being Schick tested and, if not immune, injected with toxin-antitoxin. We found that the doctors reported during the next year more than four times as many cases of suspected diphtheria among those not tested, and that all of the severe cases occurred in the untested children. Some cases were to have been expected, because only about 80 per cent. of school-children become fully immune from one series of injections.

In small cities like Auburn, New York, and New Haven, Connecticut, diphtheria has either been wiped out or nearly wiped out by the almost general use of

Results from injecting diphtheria toxin intradermally. The Park test to the left. The Schick test to the right

Dr. Schick making the Schick test—the intradermal test with diphtheria toxin—on a group of school children

The $175,000 Horse. One of our most famous "antitoxin horses," called "Old Faithful" because he produced such high grade antitoxin for so long a time

these injections. They can be given in the earliest days of life, but the best time to give the injections is shortly after the end of the first and the beginning of the second year. Babies are found to acquire before birth immunity from their mothers if they are immune. This is true for other diseases; that is, a mother who is immune to scarlet fever or to measles or the many other diseases, bears an immune baby. As the baby reaches the age of six months, nine months or a year, the immunity lessens or disappears. It would be perfectly safe to immunize babies at six months of age, but they are liable to so many illnesses that it seems best to wait until the baby is at least nine months old, because many of their ills may be attributed to the toxin-antitoxin injections. The easiest time for the public health agencies to immunize children is while they are at school, because here they are massed in large numbers. The objection, of course, is that school-children have lived to an age when the danger of diphtheria has been greatly lessened. It does great good, however; in the first place, it prevents a great deal of mild sickness from diphtheria. Again, it prevents their taking diphtheria home to their little brothers and sisters; and, finally, it educates them and their families as to the harmlessness of the injections and their value. We must depend, however, in the long run, on the parents and physicians seeing to it that the little children, those of pre-school age, are immunized.

As we look forward into the future we see the fight between the human being and the troop of diphtheria bacilli ending in the conquest of the diphtheria bacilli. The sooner every child is immunized, the sooner will this be accomplished.

## CHAPTER XIII

## THE GROUP OF THE ACID-FAST BACILLI

(Mycobacterium)

Bacilli causing tuberculosis and the "unclean disease," leprosy —The glanders bacillus.

The members of this famous group of bacteria are distinctive in their staining qualities. As their common name indicates, when once stained—and they are hard to stain—they have the power to retain this stain even though they are washed with an acid solution which quickly removes the stain from other bacteria. They are acid-fast or hold their color or stain tenaciously against acid. This characteristic of acid-fastness has grouped them together as a genus (mycobacterium) in our false-branching family, the same one to which the diphtheria bacillus group or genus belongs. But they must be thirty-second cousins if related at all. This false-branching family is called Mycobacteriaceæ, meaning fungus-like bacteria. Its name indicates the opinion classifiers have of the close relationship of this family to the molds.

The head of the group or genus of the acid-fasts is the dread tubercle bacillus, a germ that has probably caused more misery, sickness and deaths in civilized people than any other. It is a thoroughly bad one. In its wide-spread frequency, its insidious attacks of people and lower animals under civilization rather than in the

wilds, its slowly but surely fatal course in a comparatively high percentage of the humans it attacks, its mysterious mode of attack and of its host's counter-attack, its ability to grow in all tissues producing protean manifestations of disease—in all of these characteristics it is a microbe worthy of our best efforts to know it and control its ravages.

Although Villemin had shown in 1865 that tuberculous tissue when ground up and injected into animals would produce tuberculosis and therefore proved that the disease was communicable, and that the germ was in the tuberculous mass, yet many years were to elapse before the germ was discovered. It was in 1882 that Robert Koch solved the mystery. Staining methods had greatly improved since Villemin's time and Koch took advantage of these changes. He found that if he smeared a drop of sputum on a glass slide, dried it in the air, then passed it through a flame sufficient to coagulate the albumin and fix it fast to the glass, placing this in an almost boiling solution of fuchsin and carbolic acid (phenol), everything on the slide was deeply stained. Then he made his discovery. He immersed this slide in a bath of alcohol and dilute hydrochloric acid and washed out all visible color. When he placed the slide under a highly magnifying lens he was surprised and delighted to see little bright red rods. These were the tubercle bacilli, which, as we said, are hard to stain and equally hard to decolorize. This staining method is made use of in helping to detect these bacilli in sputum or in other secretions or tissues of the infected host.

Koch was also successful in cultivating the tubercle bacillus. He removed aseptically a piece of tuberculous

tissue and planted it on coagulated blood serum. After a number of days little gray colonies developed on the surface which gradually became thick and wrinkled. When these bacilli were examined chemically it was found that they contained a large amount of wax-like substance. This it was that interfered with the staining by ordinary methods and made it difficult to decolorize them after they were stained. When these bacilli from the culture were injected into animals they developed tuberculosis. This isolation of the tubercle bacilli by Koch was one of the most important discoveries ever made. It became possible to attack the disease far more intelligently.

When Koch discovered the tubercle bacillus in tubercles not only in tuberculosis in man but also in that of birds and cattle, he at first thought that the bacilli were the same in all animals. After further study, he and others were able to prove that the tubercle bacillus of birds was chiefly dangerous to birds; that it occasionally infected pigs and possibly human beings, but the latter were rarely if ever attacked by the bird strain or type of tubercle bacilli. For a time it was still thought that the tubercle bacilli which attack human beings and cattle were alike. Further study by Theobald Smith, one of the best known of American bacteriologists, discovered certain differences between the tubercle bacillus obtained from a cow and the tubercle bacilli obtained from a human. Robert Koch then tested the type of bacilli found in adult human beings. He discovered only the human type and gave it as his opinion that the bovine type was of little or no importance in man. This opinion was not accepted by all and a series of investigations were started in

England, in Germany and in the city of New York. This last investigation was carried on in our laboratory. Fortunately, these three groups of investigators came to the same conclusion. They agreed on the fact that the bovine tubercle bacilli were the only type of bacilli that infected cattle. This type was, however, more widely distributed, for it was found that it also infected hogs and, most important of all, it infected infants and children. It was found that infants and very young children developed general tuberculosis when infected with the bovine bacilli, and this took place almost as readily as from the human bacilli. It was almost always fatal. Older children developed only local tuberculosis such as in the lymph glands of the neck. Adults, as Koch had earlier found, were free from bovine infection. The outcome of the investigations, therefore, was that practically all human tuberculosis after the age of childhood was due to bacilli of the human type. A controversy then arose as to whether the absence of the bovine type in adults was due to the fact that original infection was with human bacilli or that the bovine bacilli had caused latent infection in childhood but because of remaining in the human body so long had gradually adapted themselves to the human tissues and taken on the characteristics of bacilli of the human type. It had already been noted that the majority of children in cities become infected before the age of puberty, and that much of adult tuberculosis was due to the breaking out of this arrested focus.

Careful study of material in which the bovine bacilli had remained in human tissues for three or four years, and of human bacilli which had remained in cattle for

at least one or two years, showed no changes in the two types during their stay in human beings or cattle, so we believe that although conceivably under peculiar conditions there may be an occasional slight change in the types of bacilli, yet practically all human tuberculosis of adult life is due to an original infection with the human type from a human case and has no relation to milk or any other food product.

Let us now go on to another aspect of tuberculosis. Many years ago a young man, who became one of the best beloved American physicians, Dr. E. L. Trudeau, discovered that he had tuberculosis. He had just finished his hospital work as interne and was married and ready to start on his practice. He did not feel as well as he thought he should and, seeking an examination, found that he had tuberculosis. At that time, for some reason, tuberculosis was an even more serious disease than it is at present. The conditions found in his chest on a physical examination seemed to condemn him to an early death if he remained in the city. After consultation with some of his best medical friends, he decided that his only chance was to leave New York and go up into the North Woods, where in the clean embracing air he could strive to so build up his resistance that the germs of tuberculosis would fail to progress. Although he never became completely well, his disease became arrested. He decided to try and help other consumptives to achieve equally good results. In order to bring home to others the value of good air, rest and good food in increasing resistance to the invasion of the tubercle bacilli, he planned the following experiment:

He selected a number of rabbits for the demonstra-

tion. He injected each of them with a similar amount of tubercle bacilli. Some of the animals he placed in the cellar, which was fairly light but not especially well ventilated. The others he placed in a well-sheltered porch where they were in access to the sunshine and the fresh air. To the first lot he gave the ordinary rabbit food ration; to the second lot he gave a special diet, selecting for them the food which they most desired and was best for them. The rabbits living in the cellar all developed fatal tuberculosis. The rabbits living under the best conditions, with access to the open air and the sunshine, recovered without any signs of permanent infection. Since then all the world has come to realize that while probably every member of the human race living in crowded communities is liable to infection from tubercle bacillus, yet we can so increase our resistance that the bacillus is barely able to live in our tissues, and if it does, it is usually utterly unable to cause progressive disease. While, therefore, we have not been able to find any cure for tuberculosis and, until recently at least, no vaccine or chemical substance which gives hope of making us immune, yet we have, through increasing the general resistance of the people and often isolating those with open tuberculosis, been able to conquer the disease to such an extent that less than one quarter of the number of people die from tuberculosis as did fifty years ago.

So far as cattle are concerned, the question of tuberculosis is almost as important for them as human tuberculosis is for us, and as we depend on cattle for meat and for milk, anything which endangers their health is of great importance to us. We have with cattle the ability to handle the matter of infection in a way which

we cannot do with human beings. We can eliminate from our herds all those that are susceptible. This brings us to the study of a toxic product made by these bacilli which we call tuberculin.

Koch in 1889 discovered that in the tubercle bacilli themselves, and in the fluid in which they had grown, a specific toxic substance developed which, when given to tuberculous patients, caused a marked general reaction and also a local one of the tissues infected. There was a rise of temperature and an increase of signs of inflammation in the infected area. He found that when the beginning dose was very small he could avoid any apparent reaction, and that by gradually increasing the dose the people injected would become less and less susceptible. He hoped, therefore, that he had a vaccine which would increase the resistance to the invasion of the bacilli and so help recovery. Those of us who were alive at that time remember how in 1890 a great excitement spread among those who had tuberculosis. They all longed to try this new vaccine which was to cure them. They sacrificed everything to get to Berlin and take the supposed cure. It finally developed that the immunizing effect was very slight and only of value in incipient cases. In fact, unless the tuberculin could be used with the greatest care it produced far more harm than good. Something of great value, however, had been determined—namely, that the injection of even tiny amounts of tuberculin caused a specific reaction which could be utilized to detect the earliest stages of infection in both human beings and cattle. This is given in different ways. If a small amount is injected into the skin it causes in any person or animal that has any tuberculous tissue a local redness and

swelling. A similar effect follows placing a drop of a dilute solution on the mucous membrane of the eyelid. If we have a taint of tuberculosis in our body the mucous membrane becomes inflamed. The skin reaction is absolutely harmless. If, however, we wish to be certain whether or not an animal has tuberculosis, we use a subcutaneous injection with a larger amount. After about six hours the temperature begins to rise, and hour by hour over a period of eight or ten hours it rises and then falls. Such animals are known to be tuberculous. They are removed from the herd, slaughtered, and if markedly tuberculous they are put in the rendering tank and the various chemical substances in their bodies separated and used for various commercial products. If only a tiny amount of material such as a lymph gland is tuberculous and this can be entirely removed, they are used for food. The farmer is reimbursed by the State or the Federal Government for the full amount of money which the cow was worth. In this way millions of dollars have been spent to eliminate tuberculosis.

In spite of no worth-while success, the effort to produce a vaccine has been continued down to the present day, and at the moment we are using with some hope of success a vaccine developed by Calmette, who is the assistant director of the Pasteur Institute in Paris. For years he has been attempting to immunize animals with attenuated living tubercle bacilli. After having tested monkeys and calves and rabbits, he began five years ago to give it to infants; at first to those born of tuberculous mothers, then to those born in tuberculous families and now to all infants. His method is to give the baby during the first ten days of life three feedings,

each of which contains about a half billion attenuated bacilli. The baby shows no apparent ill effect. This vaccine has been given to over 100,000 infants in France alone. He believes that the results give proof that the vaccine has immunized the great majority of them. The death-rate of untreated infants from tuberculosis during the first year of life in tuberculous families in France is about 20 per cent., while in those receiving the attenuated bacilli it is only 1 per cent. Our laboratory has been trying it out through one of our co-workers, Dr. Camille Kereszturi. She, with two devoted nurses, has during the past two years fed the vaccine to over 150 infants. Only two of these have died of tuberculosis. The first one was a premature baby, that died in its third month. This was undoubtedly a case of congenital tuberculosis. Its mother died from generalized tuberculosis at the time of giving birth to her child. The other was a fine child which was perfectly well until it was five months of age. Then it developed whooping-cough and soon afterward signs of tuberculosis. The disease progressed to a fatal termination. This child was not separated from its mother for the first month, contrary to Calmette's advice, so as to give time for the development of immunity from the vaccine. Both its parents had tuberculosis. The sputum of the mother contained many bacilli. All the living babies are well. The enthusiasm of the French leads us to hope that Calmette is right. We are only certain the vaccine is harmless, and we are looking forward with great interest to watching for the next year or two the results of the treatment. Subcutaneous injections are now being used. These seem to be harmless and are more effective.

An important relative of the tubercle bacillus is the leprosy bacillus or Mycobacterium lepræ. This acid-fast non-motile little irregular rod, very like the tubercle bacillus, probably never has killed anything like the number of people that the tubercle bacillus has, but the terrible nature of the disease it causes has made its activities dreaded almost as much, perhaps more in earlier times. Through ancient literature are many accounts of the pitiable condition of those afflicted with it. The fear of infection made them outcasts. "Unclean! Unclean!" they were compelled to cry if any one approached them.

It is interesting that it was probably in connection with leprosy, which is not highly contagious, that the first ideas of infection became implanted, and that strict laws of segregation such as those given in Leviticus were promulgated. No doubt other diseases with skin manifestations were included in those times under the term "the unclean disease." The enforced isolation of this type of disease led to a marked diminution of cases in those areas, and now in many countries of the world leprosy has practically disappeared.

It is estimated that there are now only about two or three million cases in the whole world, of which the United States has about a thousand.

We get a brilliant picture of the microbe if we stain a spread of infected tissues by the method used to detect acid-fast bacteria. Blue-colored tissue cells are filled with the brilliantly stained red rods.

And yet the question of how to grow these leprosy bacilli is still not definitely solved. We are not even sure that a true leprosy bacillus has been cultivated. The reason we can't be sure is because the lower ani-

mals are not clearly susceptible to this disease and we have no specific test to indicate that we have the right culture. A number of kinds of partially acid-fast cultures have been isolated, and some workers have claimed that they can produce leprosy in monkeys with them by repeated doses. There is no satisfactory curative treatment for leprosy.

As with all other groups of microbes there are a number of harmless unimportant relatives of these harmful acid-fast bacteria. They derive their interest chiefly from the fact that they might on occasion be confused with the harmful ones. Thus there are certain forms that grow on grasses and so may get into butter, milk and cheese. Then there are forms found in cold-blooded animals that do not seem to be at all harmful for man, in certain fish, snakes and turtles. A culture obtained from turtles is one that was used by the German Friedman to make a vaccine that he claimed would cure human tuberculosis. Like many similar claims for other so-called remedies, it proved to be ineffective.

A very distant relative of the tubercle bacillus is the glanders bacillus discovered by Loeffler in 1882. It belongs in the "false-branching" family but is not in the acid-fast group, so it is considered as belonging to a distinct genus and is given the specific name Pfeifferella mallei. It causes a well-known and earlier rather common disease of horses, asses and mules, characterized by the formation of small nodules chiefly in skin and mucous membranes that may ulcerate. This disease is known as glanders, or farcy. It may be transmitted to man and cause his death. Now, with the substitution of horses by the automobile in cities, and with the elimination of the common drinking-fountain, glanders has

practically disappeared. This microbe is like the tubercle bacillus in its ability to produce a substance similar to tuberculin called mallein. It gives a reaction in animals affected with glanders comparable to the reaction of tuberculin in animals affected with tuberculosis. Injections of it are used to detect hidden glanders. While the disease is now infrequent, we should be on our guard against it in any places where many horses are kept.

## CHAPTER XIV

## THE COMMA FAMILY AND CHOLERA

(Spirillaceæ)

In the early nineties the words Asiatic cholera were still causing thrills of dread, especially in the minds of those who were traveling to Europe. For at that time the last great epidemic of this terrible scourge was still raging, and a devastating and, at first, unexplainable outbreak had just occurred (1892) at Hamburg. This whole epidemic extended over a dozen years. At its beginning, 1883, in order to investigate its cause a cholera expedition headed by Koch was sent to Egypt, where the disease had broken out in force.

Long before this time water was suspected of being the chief medium for conveying the cholera "virus" to man in epidemic form. Indeed, as early as 1854 Snow of England practically proved that cholera followed the drinking of water that was exposed to the dejecta of cholera patients. Many agreed with him that cholera was a specific infection due to a special virus, but others still thought that a group of general conditions was necessary to produce an epidemic. Indeed, even after the discovery of Koch's comma bacillus, authorities such as von Pettenkofer and his followers believed that the receding of the ground water in a locality was essential to the breaking out of an epidemic.

At this time, when our knowledge of microbes was

still in its infancy, there were many other mistaken ideas that different people had of the conditions, including that of the hand of God, which they thought caused or helped the outbreaks of cholera and other scourges.

Among them was the idea, held until very recently of most communicable diseases, that the air about the patient contained an effluvium given off from the patient that caused the disease if one breathed it in. A description of the results of the brutal and dangerous custom of allowing people to die uncared for and alone because of this idea would fill many a chapter of history of the world's sorrows.

It is chiefly the work of Snow and his like, who were true logical observers, that made a change for the better, as I said, long before the final proof came.

Snow insisted that the characteristic rice-water stools of the cholera patients were the chief, if not the only, site of the cholera poison, and that these discharges reaching the drinking-water constituted the chief means of the spread of an epidemic. Of course, he recognized that there might be other ways also for the poison to be communicated.

Newsholme[1] calls attention to an absurd (to us now) incident that occurred in Scotland at the time the Broad Street pump epidemic started. He states:

"In 1853, when cholera, which had previously devastated Great Britain, was once more threatened, the Presbytery of Edinburgh wrote to Lord Palmerston, then Home Secretary, suggesting that 'in the circumstances a national fast should be appointed on royal

[1] "Evolution of Preventive Medicine," The Williams & Wilkins Co., Baltimore, 1927, p. 10.

authority.' In his answer the Home Secretary made a statement which shows the realization of the fact that epidemics are governed by natural laws. He wrote: 'The weal or woe of mankind depends upon the observance or neglect of these laws.' "

He further emphasized that activity in the direction of purification of towns was preferable to humiliation, adding:

"The best course which the people of this country can pursue to deserve that the further progress of the cholera should be stayed will be to employ the interval that will elapse between the present time and the beginning of next spring in planning and executing measures by which those portions of their towns which are inhabited by the poorer classes, and which from the nature of things must most need purification and improvement, may be freed from those causes and sources of contagion which, if they be allowed to remain, will probably breed pestilence, and be fruitful in death in spite of the prayers and fastings of a united but inactive people."

In St. James parish, where the famous Broad Street pump was situated, 700 people died of cholera within seventeen weeks. Snow, who had long thought that infected water was the cause of cholera, called attention to the fact that the great majority of these cases occurred within the area supplied with water from this pump. Few records were kept in those days, and the people were ignorant of health makers and non-observant, so Snow had great difficulty in getting clear-cut information; but he persisted in his investigations, even giving up his practice for a while in order to have time to visit personally the families in which cholera

Making the Calmette vaccine that is being tried for its protective worth against tuberculosis

Protecting an infant from tuberculosis. The doctor having put the required amount of the Calmette vaccine into the milk is going to feed it to the baby.

had occurred. In his search he unearthed a number of significant incidents which together formed an impressive mass of evidence in favor of the town pump of Broad Street being infected with cholera virus. One or two may be cited:[1]

There were many town pumps at that time, but according to Snow the Broad Street pump seemed to be a favorite one. One woman who lived at a distance from the pump had a cartman bring the bottles of water from this well occasionally, because she thought it so good. Just at the time that the epidemic broke out he had brought her some. A niece, living in an area where they had no cholera, was visiting her when the water arrived, and they both drank of it freely. Within two days they each came down with a fatal attack of cholera.

A man living near the pump and using its waters was stricken with the cholera. He sent for his brother, who lived in a non-cholera district. The brother arrived after the death of the man and was not allowed to see the body. He drank some of the water, however, before he left for home. That night he came down with cholera and quickly died. No other cases of cholera developed in that place.

Snow showed further that while a certain factory in the neighborhood that had a well of its own had no cases of cholera, another near-by that kept two tubes of drinking water drawn from the Broad Street well had many cases of cholera.

Snow examined the surroundings of the Broad Street well and found a deplorable condition. From a near-by

[1] See Rosenau's very good description of the epidemic in his "Preventive Medicine and Hygiene," 1927. New York: D. Appleton & Co.

house went a defective drain to the sewer, passing the well within three feet. Near the well also was a cesspool, over which was a privy used by people of this same house. In this house there must have been an earlier unrecognized case of cholera. Later four cases of cholera developed there.

When the Broad Street well was demolished the cases of cholera in that neighborhood ceased.

Notwithstanding Snow's striking array of evidence, proof of the important part played by water in outbreaks of cholera had to wait until some time after the discovery of the little curved bacillus, comma shaped, that Koch first found in the dejecta of cholera patients in Egypt.

When Koch saw under the microscope myriads of minute curved germs and scattered spirals moving rapidly to and fro with vibrating motion in the fluids obtained from the stools of cholera patients, and when he could not find similar ones in normal stools or in those from other diseases, he was sure that these tiny wigglers were the cause of cholera, though he hadn't yet obtained a pure culture of them. He said, "Send me to India, the home of cholera, and I will prove to you whether or not these are the microbes that are doing the mischief." So he was sent to India. There he found the same kind of a germ in the cholera cases and did not find them in others. He also found them in a stagnant tank of water which people coming down with cholera were drinking. And better yet, he obtained a pure culture of the micro-organism. He called this the comma bacillus or the comma vibrio. It was also called the cholera spirillum. It is in every sense of the word the

chief of the group of microbes composing the family of spirilla or comma-shaped germs.

Koch had great difficulty at first in completing the chain of evidence that would prove his contention that the microbe he found causes cholera. This was because the organisms, though growing so abundantly in the human intestinal canal, could not be made to produce symptoms like cholera in any of the lower animals or even give evidence of growing in their intestinal canals. He found later that the cholera vibrio does not like acid even in traces. In fact, it has been found that they are quickly destroyed by weak acids. It was then suspected that when animals were made to swallow them the acid in the gastric juice killed them before they could reach the intestines. After Koch found this out, he succeeded in producing symptoms and intestinal lesions in guinea-pigs similar to those in many humans by introducing large amounts of the cultures through the mouth by catheter after first neutralizing the stomach contents with a solution of bicarbonate of soda and inhibiting peristalsis of the intestines by the use of opium.

In the meantime, one of the earliest accidental infections of laboratory workers furnished more satisfactory if more tragic evidence that the cholera vibrio is able to produce cholera in man.

In 1884 a student studying the cholera germs in Koch's laboratory in Berlin became acutely ill with a typical attack of cholera. At that time there were no cases of cholera in Germany. The organisms were found in his rice-water stools.

It seemed as if the vibrios were able to pass the gas-

tric juice guard of man more readily than that of most of the lower animals. Man is probably also much more susceptible to infection than animals. Having once passed that guard, Koch claimed they were usually able to multiply exceedingly in the alkaline medium of the intestinal contents, which suited them very well.

It was shown later that Koch was right. When a few pass the stomach into the intestines they soon may overgrow the normal inhabitants of the intestines, especially in those people who are suffering from indigestion. In their growth they produce a toxic substance that makes the intestinal wall red, swollen and hemorrhagic. They cause their victim to have violent vomiting and diarrhea which take away so much fluid from his body that the patient may quickly look like a wrinkled old creature—a veritable mummy. If saline infusions are given at this stage the patient's change back to a normal appearance may be remarkable. But alas! in at least one half the cases this change is not lasting. In these cases the diarrhea and vomiting continue, the patient passes into a collapse and quickly dies. The comma vibrios are found in almost pure cultures in the characteristic rice-water stools of these cases.

Koch's inability to demonstrate clearly and quickly a chain of evidence proving the etiologic relationship of the cholera vibrios to cholera allowed the doubt to grow in many people's minds as to the specific nature of the vibrio in its relation to cholera.

Two men who were among the unbelievers said they knew they could swallow masses of cholera vibrios without developing cholera afterward. So, having first taken some sodium bicarbonate to neutralize the acid in their stomachs, and some opium to stop the peristal-

sis of their intestines and keep the organisms from being hurried through, they each swallowed about a teaspoonful of a fresh vigorous broth culture of the vibrios. Von Pettenkofer, of the ground-water theory of cholera epidemics, was one of these men. Within a day or two he came down with a severe attack of diarrhea from which he recovered in a few days. His companion, during the night following the injection, became very ill, he had severe diarrhea, cramps and vomiting, with great prostration. His stools became typically rice-water. This condition lasted several days. Then he gradually recovered. Clinically he had had a typical attack of cholera. The cholera vibrio was recovered from the stools of each man.

Other laboratory workers repeated this experiment, using themselves as the test animals—some came down with typical cholera and some did not. The fact that some did not was explained by the assumption that these had a higher degree of resistance than others. Then another student, working in Hamburg with cultures, came down with the disease and died.

On the whole, these human experiments and the accidental infections made the evidence so strong that the case for the cholera vibrio being the specific criminal in cholera epidemics was considered won, at least by the majority of research workers.

Then came the crowning evidence. This was gathered during the fatal outbreak of the disease in Hamburg in 1892. The clear-cut results from the investigation of this evidence form one of the most instructive and convincing demonstrations we have ever had of the relation between the dejecta of cholera patients, the cholera vibrio and the water supply with its control.

It was during the time the great epidemic referred to at the beginning of this chapter was nearing its end that this tragic outbreak occurred.

Cholera smolders in Asia chiefly along the Ganges and in Lower Burma. Why it remains endemic, forming hotbeds in certain areas, no one clearly knows. Perhaps there are more chronic carriers in these areas than investigators have been able to demonstrate.

This last world-wide outbreak was said to have been started after a great festival along the river Ganges, in whose waters many people bathed as a part of the rites.

As the epidemic spread over the continents it manifested itself with marked intensity in Russia. It is estimated that in Russia alone, during the dozen years these outbreaks continued to occur, the vicious little comma vibrio claimed over 800,000 victims.

It was to be expected that an epidemic might spread with great vigor in countries like Russia, because of the wide areas not under hygienic control. But in those parts of the world that were able to carry out rules of hygiene it was taken for granted by the beginning of the nineties that enough was known about cholera to prevent its spread in such areas.

They knew the cholera germ was in the dejecta of patients. They knew that this germ might be carried by the water as well as by direct contact. They knew, or thought they knew, that if the dejecta of patients were disinfected, cholera would not spread.

And then came the sharp Hamburg outbreak. The authorities were filled with consternation. They couldn't understand it. That such a thing should

happen in such a well-conducted place in one of the headquarters of culture and hygienic efficiency!

From the middle of August to the middle of October 1892 there developed about 17,000 cases. Death followed death—fully half of the cases were fatal. In the near-by city of Altona there were scarcely any cases.

Investigation brought to light the deplorable fact that while Altona was using filtered water from the river Elbe, Hamburg was using the raw unfiltered water from the same river.

Further search showed that the epidemic was practically confined to those using this unfiltered water.

But they cried, "How did the water become infected? There were no cases of cholera along the Elbe just before the outbreak." That the water was infected was proved by Koch's finding the comma vibrio in it. Then they found the probable source of the outbreak among a number of Russian immigrants from infected areas, waiting in barracks along the Elbe to be shipped to the United States.

The authorities didn't find any pronounced cases of cholera among them when examined, but they decided that there must have been some undetected light cases or recent convalescents. At that time it had not been determined that healthy people might carry the germs. No doubt there were several of the different types of carriers among these Russians, and they constituted the first source of the pollution of the Elbe. Then, as other cases came down, all emptying their sewage into the Elbe, the outbreak grew in intensity.

When the inhabitants began to boil their water, and

when they established an efficient filtering plant for the river water, the outbreak stopped.

This epidemic might almost be called a pandemic. It has been called so by some; for a few cases, at least, appeared in almost every country in the world. In the United States there were several cases, but owing to the vigorous inspection and quarantine of incoming steamers, and to the fact that no water supply became infected, the disease did not assume epidemic proportions in this country.

An interesting incident occurred in New York City at that time in connection with the outbreak of cholera.

Dr. Dunham, who was one of our noted pathologists and who was the first American to study cholera, late one night performed an autopsy on a case of cholera that had died at the isolation hospital of New York City. He gave his trusted helper Toby some of the autopsy material in a sealed pail to carry to his laboratory in the Bellevue Hospital Medical College. As Toby was hurrying from the morgue to the laboratory through the notorious "gas-house" district he almost collided with a policeman coming suddenly around a corner. "Hi! Where do you think you are going?" called the officer, seizing him by the arm. "Look out," said the messenger. "Don't disturb this pail." "What have you got in it?" asked the officer. The messenger, who was something of a wag, replied solemnly, "I can't tell you." "You must show it to me," insisted the officer. "I cannot open it. Don't you see it is sealed?" said the messenger, pointing to the sealing labels plastered over it. "Well, then, you'll have to come with me to the station-house. We'll see if the chief can make you talk." It was too late to confess now, even if the

messenger had thought it wise to do it. So he went with the officer to the near-by station-house. When the messenger told the captain what he had in his pail, every one gasped with fear and horror of infection, and the police captain hurriedly detailed a squad of policemen to escort the messenger with this dangerous package safely to the laboratory.

It was during this investigation of cholera at this time that Dr. Dunham devised the famous solution that bears his name—Dunham's peptone solution. The cholera microbes develop in this medium the cholera red reaction. He showed that while the cholera red reaction was not absolutely specific for the cholera vibrio, all true cholera strains show it. So any strain not showing it is not a cholera vibrio. This reaction was one of the many finds that were thought to be peculiar to the cholera vibrio, but that later researches showed not to be absolutely distinctive, but still of great practical value.

During the search for cholera vibrios a discovery was made similar to that which has been found in connection with all well-studied microbes; and, as always, especially in the early days of investigation among microbes, led at first to open the doubt then existing in the minds of the few as to the specificity of the microbe in question.

This discovery was the existence in conditions other than cholera of vibrios so similar to the comma vibrios that in most instances special tests had to be developed to show that they were not identical with the cholera vibrio.

Thus, similar vibrios were occasionally found in normal saliva, others in a certain kind of cheese; others,

and many others—for vibrios like water—found in water. Then some were found in the diarrheal stools of infants and adults. So there was finally a fair-sized family of vibrios to be studied and shown up as being composed of distinct individuals.

Many were the points of difference and resemblance between these forms, brought out by the painstaking studies made of them, but little emphasis is laid now on most of these points so strongly insisted upon by their discoverers at that time. For the so-called serologic tests proved to be so specific that the others were found to be for the most part unnecessary. Of course, selective culture media must be used to get the strains easily in pure cultures. Dunham's alkaline peptone water is favorable for growth, and as these vibrios grow more abundantly in the presence of oxygen, they may be found in a short time in large numbers at the top of the medium. A small loopful taken from the upper part of this growth after six hours and planted on plates of nutrient alkaline agar medium to which a little white of egg has been added will show very typical colonies of the comma vibrio, which may be fished and planted on plain agar and fished again before we can be sure we have a pure culture. There have been many other methods recommended for obtaining pure cultures of the cholera vibrio.

Then when we are sure we have a pure culture we test it by employing the serologic test that is now always used to determine the relationship of identity between strains—namely, the absorption of agglutinins.

By this test it has been shown that the real cholera vibrio is rarely found except in cases having typical

symptoms of cholera. In the presence of an epidemic or in countries where it is endemic it may be found in less typical cases and in an occasional normal person.

Another specific test that was early brought out by Pfeiffer may also be used, but it is more cumbersome and it is not as clear-cut as the agglutination test. This is the so-called Pfeiffer's phenomenon or the bactericidal test, which we have already described. This test was shown for the first time in connection with studies on the cholera vibrio.

Pfeiffer found that the serum of animals that had been immunized against cholera contained a substance that together with the peritoneal fluid would kill and then dissolve the cholera spirilla when mixed with them. Such a serum had very little if any effect in curing the cases, but it might prevent the disease if given before the infection.

This led investigators to try preventive vaccines in face of an epidemic and in those countries where the disease is endemic. As a result they have found that a vaccine does have marked protective action in a large percentage of the cases. It is of almost as much value as the typhoid vaccine against typhoid fever. Any one about to visit a country where cholera is endemic should take this vaccine.

## CHAPTER XV

## THE COILED-HAIR FAMILY

(Spirochetaceæ)

The pale spirochete and the immoral disease—Spirochetes in relapsing fever and in yellow fever.

As long ago as 1833 a German named Ehrenberg, while examining some water under the microscope, saw a number of squirming creatures that looked like living motile coils of hair. Because of this appearance he called them spirochetes. Since that time many similar germs have been discovered, some in stagnant or fresh water, in the sea, and one variety even in hot springs; some growing as harmless parasites in animals, others invading their hosts as deadly enemies. No known spirochete has so far given evidence of being useful to man.

Among the known harmful microbes, perhaps the one that has done the most all-around damage to human beings, from the disturbing of domestic life to the ultimate killing of its victims, is the chief poisoner of this group, called familiarly by the unassuming name, the pale spirochete.

According to the custom of classifiers, its scientific name, which was Spirocheta pallida, has been changed to Treponema pallidum. The reason for making this change is the usual one. The classifiers decided that this spirochete had characteristics so different from those

of the then known spirochetes that a new genus should be created for it. They reported their claim and gave their genus the name Spironema. Finding that this name had been preëmpted for a class of mollusks, they chose the name Treponema, which means a turned or turning thread.

And so the pale spirochete, or pale turned thread, at present must be introduced formally as Treponema pallidum, the chief of its genus. This arch criminal, even more than other varieties that have been placed in its group, is made up of extremely delicate translucent material. For this reason it remained hidden from the searching eye of the microscopist until only a comparatively few years ago.

Though it insinuates its way into nearly all of the tissues of those unfortunates who have become infected with it, even crowding their lymph nodes, livers, spleens and other parts of the body at certain stages of the terrible protean venereal disease that it causes, yet it was missed completely by all of the bacteriologists who had been eagerly hunting for the cause of syphilis, as the disease it causes is called, ever since microbes were discovered and microscopes were perfected.

"The history of the search for the cause of syphilis is a tale to make the judicious grieve," says Osler.[1] One hundred and twenty-five claims made in the twenty-five years before 1905, each of a different cause, are quoted by Lasson.

Finally, in 1905, two biologists, Schaudinn and Hoffmann, who had made protozoa their special study, began to investigate the cause of syphilis. Soon they found something that filled them with hope. In ma-

[1] Osler, "Modern Medicine," 1907, vol. iii, p. 440.

terial scraped from a primary mucous sore on the lip of one of the men under examination they saw through their immersion lens some extremely delicate spirals that occasionally made a slight jerking motion.

"Here is a new microbe," said one of the observers. "They may be only tissue fibrils," said the other. "No, that can't be," said the first; "that is the motion of a living thing, not merely of an irritated tissue fibril." So they continued to study the thing. They examined the discharges from other people showing symptoms of the disease and they found the same peculiar thing in each one. They at last decided that it was really a new microbe and the cause of the disease. And later studies of it proved conclusively that they were right.

While these delicate living spirals were distinguished under an ordinary immersion lens by the two keen-eyed observers Schaudinn and Hoffmann, it was not until they were examined on a dark field stage whose brilliant side lighting illuminated very minute and delicate forms that they could be seen with any degree of clarity by the ordinary examiner. So their discoverers deserve all the more credit for finding them with the help of only the ordinary immersion lens.

Another reason why they were not found before is that they stain so faintly. Their discoverers tried many staining methods, none of which made the spirals appear very distinct. The stain that brought them out most clearly is a complex one known in its various forms by the different names of its developers. The mixture used by Schaudinn and Hoffmann for staining the Treponema pallidum is known as Giemsa's stain. It was a favorite stain for bringing out the structure of protozoa. It is made up of a mixture of blue stains

(methylene blue and methylene azure) and of a pink stain (eosin), and it brings out the structure of protozoa very well, but it only makes the syphilis spirals take on a faint azure stain. However, it differentiates them from most of the harmless spiral forms that are found in the mouth. These take a bluish color by the Giemsa stain. Later, a method of silver impregnation of these microbes was devised that shows the pale spirochetes most spectacularly even in the tissues where they appear by this stain as jet black spirals on a pale gray background.

The announcement that a new microbe had been found in that mysterious disease syphilis, that at last the probable cause of the disease had been discovered, was received with great enthusiasm, and many research workers started out to become better acquainted with it.

First they tried to see if it would grow outside of the human body in pure cultures, just as most bacteria do. But though they gave it every kind of food they thought it might like, it wouldn't grow for them. It gave no sign of multiplying.

Just before the discovery of this spirochete, Novy and MacNeal in Ann Arbor had succeeded in getting pure cultures of a blood parasite belonging to the protozoa, a flagellate relative of the microbe that causes Oriental sleeping sickness. This was the first and, until some time later, the only protozoan that was cultivated in pure cultures.

Partly because of the apparent inability to grow the syphilis spirochete and partly because its discoverers thought it resembled certain protozoa, it was classed by them with these lowest animal forms.

But when the bacteriologists began to study it they

insisted that it resembled the bacteria more than it did the protozoa. The controversy waxed warm between the two factions. The question as to the relationships of this type of microbe is not yet settled. Read Noguchi's statement [1] regarding it in his latest (and alas! his last) [2] contribution to our knowledge of the Spirochetes.

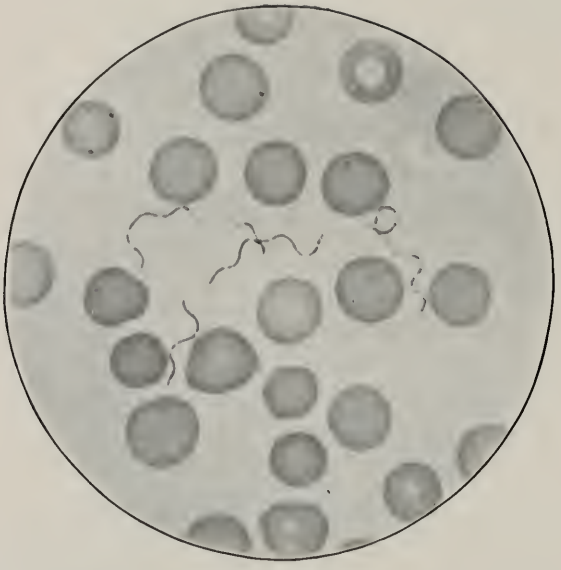
This wizard among microbiologists, Noguchi, was the one who finally succeeded in growing pure cultures of the Treponema pallidum. He found that it cannot grow when exposed to the air; in other words, it is anaërobic. He found also that it needs a bit of animal tissue in the fluid serum-water used for its growth. The animal tissue, such as rabbits' kidney, not only supplies it with food but it helps absorb any free oxygen that may be present in the fluid. Theobald Smith had called attention to this fact some years earlier when he recommended the adding of a bit of fresh animal tissue to a fluid culture medium to help secure anaërobic conditions.

Noguchi studied many different varieties of spirochetes, both in their natural habitats and in the pure cultures he was able to obtain of them. His studies made us better acquainted with the individuality of these spiral organisms and of their relationships in the family group.

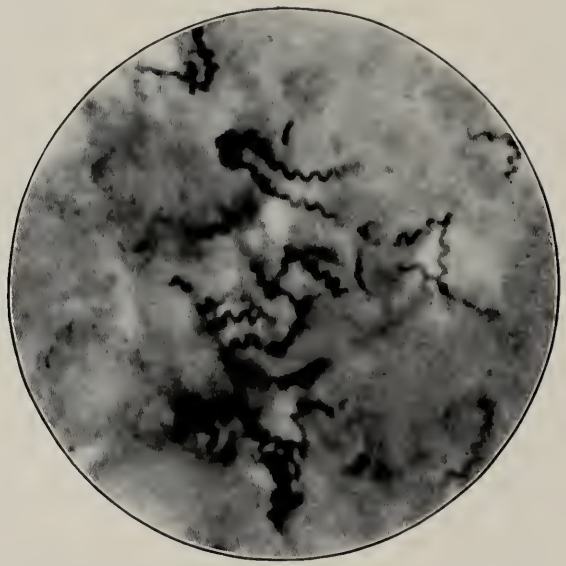
In one particular his classification does not agree with that of the Society of American Bacteriologists. He accepts only five groups or genera in the family and the S.A.B. accepts six.

[1] In "Newer Knowledge," p. 455.

[2] Noguchi died recently in Africa from yellow fever contracted while studying this disease.



The relapsing fever spirochete (Borrelia recurrentis) in blood of patient. Enlarged 1500 diameters



The "pale spirocheta" of syphilis—Treponema pallidum. Silver impregnation. Enlarged 2500 diameters

A corner of the New York City Health Department Wassermann laboratory. The chief of the laboratory is "reading" a test from the blood of a syphilitic patient

Certain varieties that Noguchi includes among the Treponemas are considered by a number of observers to be characteristic enough as a group to be given a special name. The name Borrelia after Borrel was the first one chosen for this group, so that is the name it goes by now.

The first group in this family still retains the name Spirocheta that Ehrenberg gave it so long ago. In this group the largest spiral microbes are found. They are not only distinguished from the other large forms by their size, but by having a distinct axial filament. They may be found in almost every kind of water.

The next group or genus, called Saprospira, meaning rotten or putrid coil, is found in sand containing decaying animal forms (forominifera). It is composed of large forms in which no axial filament is seen.

The third genus is also characterized by its size and by its lack of axial filament. But it has a very striking appearance. Around its body is wrapped a more or less wavy membranous structure made up of numerous elastic fibrils like a felt work of bacterial flagella. At a glance it looks something like the undulating membranes possessed by some of the flagellated protozoa. This peculiar spirochete lives as a harmless parasite in the alimentary canal or crystalline styles of oysters and other shell fish. It is called Cristaspira because of the crest on its coil.

Among the remaining three genera are the spiral microbes of greatest interest to man.

Among the species of the genus Borrelia, the most important one, from man's standpoint, though it is not the biologic chief, is Borrelia recurrentis. Here we have another illustration of the changing of the older,

original name to one of a more recent date when some new investigator establishes a new genus whose chief is an entirely different variety.

This most important microbe among the Borrelia, and the one first discovered and described, was originally given the name Spirillum obermeieri, after Obermeier, who found it as long ago as 1873 in the blood of people suffering from relapsing fever. Then classifiers decided that it belonged among the spirochetes. So its name was changed to Spirocheta recurrentis. When a new genus was created for the pallidum the spirochete found in relapsing fever was put with this genus, so the name was then changed to Treponema recurrentis. When it was decided to make a new genus of this group it was called Spironema recurrentis. Then classifiers found that a similar form, discovered earlier in a fatal blood poisoning disease of chickens, had been described by two Frenchmen in 1903 and called Borrelia gallinarum. Since this was the first time a genus was described for this type of microbe, Borrelia gallinarum became the biologic chief of the genus, and our spirochete of relapsing fever received the name Borrelia recurrentis.

Can one wonder that people have difficulty in remembering which name has finally been chosen as the right one for some of our microbes?

The disease caused by this organism is called relapsing fever because the first attack of the six-day fever is followed after an interval of about a week by another attack, and sometimes by a third, a fourth and even a fifth attack. The patient usually recovers. In India, where many cases occur, from 5 to 10 per cent. may die. There have been a great many cases in

Great Britain and Ireland, few in America and many in Africa and India. Before spirochetes were found in these various cases, the disease was classed with typhus fever. The fact that it occurs more frequently among unclean persons who live in crowded dirty quarters and that it may be conveyed by biting bugs makes a relationship to typhus apparent.

Each strain of spirochete obtained from relapsing fever in different parts of the world seems to have serologic characteristics individual enough to give it a separate specific name. So if you want to meet all the important Borrelias pathogenic in man you must know that besides Borrelia recurrentis from England, Ireland and other parts of Europe, there is a Borrelia duttoni found by Dutton and Todd in Africa, a Borrelia Novyi found by Shellack in American relapsing fever, a Borrelia berbera, found by Sergent and Foley in Algiers, Tunis and Tripoli and a Borrelia carteri, found by Carter in India. All of these are alike morphologically but differ serologically.

The same kind of a spirochete with minor differences has been found in a number of the lower animals as the cause of a febrile disease. We have already mentioned the one causing fever in chickens. It is transmitted by the bite of ticks. Another variety causes a relapsing fever in cattle, another in geese.

Then there have been varieties found in the blood of mice, in the stomachs of flies, and in the blood and ulcers of hogs suffering from hog cholera. These are no doubt secondary invaders. There are also forms found on the mucous membranes of man in association with bacteria. Most of these are probably harmless parasites.

Now we come to the point of considering more intimately that worst member of the spirochete family, the Treponema pallidum.

What volumes have been written of the activities of this microbe! How that disgusting disease it causes, the great pox, the running issue, the scab, the botch, lues, syphilis, venereal disease, to mention some of its names, is spoken of with lowered voice in the presence of the innocent. How it shocks moralists to learn of each new victim.

For this is the immoral disease. Indeed, during the fifteenth and sixteenth centuries, when immorality was so rife, when chastity was considered harmful and license was encouraged, when wars and crusades helped its spread, it became a veritable malignant pandemic. All were said to be infected with the "bad disease," with venereal diseases, for gonorrhea or gleet was included with syphilis. And no one then knew about the nature or cure or prevention of these diseases.

It was during this time that the name syphilis came into use.

That famous Veronica physician, Fracastorius, who was one of the first to speak of diseases being contagious, wrote, in 1530, a "poem" about a swineherd whom he called Syphilis, from two Greek words which mean, I love a sow. In this he declared that this swineherd Syphilis was the first person to have this dreadful pox, and that it was inflicted upon him by God in punishment for his blasphemies against God as the supposed author of a deadly blight among the herds Syphilis was tending.

The real origin of the disease is lost in antiquity.

As knowledge grew of how the disease was trans-

mitted and how it might be prevented, its ravages among the more enlightened became somewhat restricted, but even now, when we know definitely how to prevent it, it is still one of the most wide-spread of human ills. In those parts of the world like the United States, where it is less frequent, it is estimated that at least one tenth of the population is infected with Treponema pallidum, and in those parts of the world where public health has not been developed into an efficient social activity the number of inhabitants estimated infected is put as high as 60 per cent.

This lack of control of the pale spirochete is due chiefly to the fact that the measures recommended for stopping its inroads are so closely associated with questions of sex, morality and alcoholism, all fundamental social questions. People often stimulated by alcohol give way carelessly to their passions, regardless of the results. Then when they come to their senses and realize that they may be infected they are ashamed to have it known.

This means that the doctor doesn't see the case until too late to effect a quick cure, and so too late to stop its spread, often to innocent victims. When one realizes that the innocent ones may be among the unborn children, who fortunately for them may be born dead, or unfortunately for them may show terrible signs of the disease after birth, or, most unfortunately, may show no sign of this insidious disease up to twelve years of age or even older—when we think of this we say, Away with shame! Either do away with prostitutes—*male and female*—those vicious links in the cycle of deterioration of human beings, or, until that time comes, for it surely will come, urge cases to report

promptly and be treated immediately. Whether the infected one be victim or culprit should have nothing to do with interfering with his having the benefit of the marvelous results from the present-day treatment.

Even if the one infected does not consult a physician immediately, while he runs the risk of infecting others, the damage to himself may not be great until much later. All through the primary and secondary manifestations of the slow progress of the Treponema pallidum through man's tissues its victim may be cured (with always the occasional exception) by one or both of two drugs. First there is mercury, one of the oldest drugs, the effects of which in syphilis have not their parallel in medicine. Then there is that wonder among drugs, arsphenamine, or the 606th preparation from arsenic that Ehrlich developed after researches that are recounted so thrillingly by De Krief in his "Microbe Hunters." Bismuth is also much used.

Of course, these powerful drugs must be given under the close supervision of experts, and their use sometimes extends over long periods of time. They attack and kill the deadly Treponema directly, and usually, when properly given, do no harm to its host.

The favorable results from treatment are determined by that complex test the so-called Wassermann reaction that we mentioned in Chapter IV. This test indicates whether or not the Treponema pallidum is alive and actively carrying on its work of destruction in the tissues. So the test is used in the first place in helping to detect infection, and in the second place to determine when the drugs used have killed the microbe.

Other tests have been used for this same purpose,

such as Kahn's and Kline's precipitin tests, which are not as cumbersome as the original Wassermann test, and seem to be just as true indicators. But before any such test is substituted for an established one, a large series of control observations must be made. This is just what we are doing now in different laboratories.

There are two chronic nervous affections of man that used to be called parasyphilis, one jumping palsy and the other a kind of insanity. When Noguchi in 1913 demonstrated in the brain and spinal cord of these cases the presence of the Treponema pallidum, it was recognized that these conditions should be added to the already protean conditions caused by this sinuous and insidious microbe. They are late syphilis of the nervous system.

At this late stage of the disease, when there may be evidences of the destruction of tissues throughout the whole body, it may be too late to get any good effects from drugs. Potassium iodide is given at this stage to help resolve the inflammatory conditions of tissue cells.

The near relations of the Treponema pallidum—that is, the other members of this genus—are few, and most of them are probably only harmless parasites on the mucous membrane of man's orifices. Noguchi was the one who first grew these in pure cultures and studied them minutely enough to name them, with reasonable assurance that the names would hold, at least until other workers could become better acquainted with them.

One of those not included as harmless parasites is so like the pallidum in appearance that it cannot be differentiated morphologically. It was found by Castellani in a disease very similar to syphilis occurring in the

tropics and known by the name of yaws, peon or frambesia. Castellani maintains that it causes the production of specific antibodies in experimental animals, and that therefore it should be considered a different species. It is called Treponema pertenue.

A Treponema very like the pallidum has been found in a spontaneous venereal-like disease of rabbits. This is a very important discovery from the standpoint of the research worker, since rabbits are used so often in studies of the pallidum. However, Noguchi has pointed out differences between them which seem to differentiate them. He gave it the name Treponema cuniculi.

A Treponema was found in pond water by Wolbach and Binger that was shown by them to be filterable. Novy and Knapp had shown a half dozen years before that the Borrelia may pass through a stone filter. The question of the filterability of this type of microbes is of special interest since the finding by Noguchi of a spirochete in one of the dreaded diseases of the tropics, yellow fever. For a time it was considered to be the cause, but now it is thought to be only a secondary invader, and the real cause of this disease is again attributed to a filterable virus.

It is interesting to recall that two of the biggest scourges of mankind are among the diseases attributed to this type of microbe, and that both were brought practically under control before the organisms said to cause them were discovered—one, syphilis, by the power of mercury; the other yellow fever, through the wonderful work of the American commission which will be described in the chapter on filterable viruses.

The spirochete found by Noguchi in yellow fever

is the most minute among these forms. It has tightly set regular shallow spirals, very similar to the forms found a short time before by two Japanese investigators, Inado and Ido, respectively in the two disease infections jaundice and seven-days fever. Noguchi considered that a new genus should be created for the three forms, for which he proposed the name Leptospira, which means fine coil. He called this new spiral microbe, which he thought was the cause of yellow fever, Leptospira icteroides. Then he found that Stimson, way back in 1907, had found a spirochete in the blood of yellow fever patients and named it Spirocheta interrogans. Noguchi, after examining Stimson's preparations, came to the conclusion that the spirochete he saw in them was probably the same as the one he had just found. These tissue spirochetes have a way of hiding themselves that makes them hard to find. This is the reason, said Noguchi, that no one had found them in yellow fever in the meantime.

So the name of this spirochete also is changed. It is now known as Leptospira interrogans. The name interrogans is descriptive of the characteristic hooked ends, like interrogation marks.

Noguchi studied his spirochete in many ways, but notwithstanding all the evidence he brought forward in favor of his spirochete being the culprit in yellow fever, the majority of other observers who have followed his work with interest and appreciation consider that it is probably only an adventitious invader.

Indeed, Noguchi himself was in doubt as to its being the cause of the disease called yellow fever in other parts of the world. And the results from the recent

investigations of African yellow fever, including the tragic death of Noguchi there from the disease while studying it, have all given evidence that yellow fever, at least in some parts of the world, must still be listed with filterable viruses.

## CHAPTER XVI

## THE BRANCHING FAMILY

(Actonomycetaceæ)

The lumpy-jaw microbe—Infrequent but insidious attackers of human beings—Decomposers of organic matter.

As a probable link between the bacteria and the molds the branching family occupies an important place in the considerations of the systemic classifiers. The tendency of its members to form branches as do the molds is a significant characteristic that favors the idea of close relationship to this higher class of plants. On the other hand, the fact that their slender branched filaments break up freely into unbranched bacilli and coccal forms looking exactly like the bacteria is in favor of their closer relationship to these lower plants. When those that invade man are first found growing in the body fluids they usually present this appearance of small bacilli or cocci, hence if not isolated and studied in pure cultures they may be mistaken for lower bacteria. But in their mass appearance in cultures many of them resemble strongly the molds. They make a tough dry wrinkled growth on the surface of the food medium that sends tenacious strands down into the depths of the medium where they may become firmly attached.

Sometimes their surfaces are covered with the down of aërial branches. Some of them form end spores more

like certain molds than bacteria. In fluid media they form fluffy white balls that look very much like most molds. It certainly looks from this description as if Castellani and others were right when they placed those forms with the molds.

Not only is their family position in dispute, but the relationships of the cultures obtained from different sources are still largely unsettled. This puts us in more doubt than with most groups as to what we should call them.

We know we shouldn't call them streptothrices, because that name has been preëmpted. But shall they all be called Nocardia after the Frenchman Nocard, who first described an important microbe of this type in a farcy-like disease of cattle? Or shall they all be called actinomyces or ray fungus, the name first given to another culture of this type obtained by Harz in 1877 from a similar disease in cattle? Or shall they be divided into two groups or genera as some think they should, those producing definite spores called Nocardia and the other, in which definite spores have not been seen, called actinomyces? Or shall the name actinomyces be dropped entirely even as family name, as does Castellani? Or shall there be still other genera to mark more minute differences, as others have recommended?

These are some of the serious questions that are occupying the attention of our classifiers who are studying this group.[1]

Whatever our uncertainty at this stage of our acquaintance with them about accepting generic and

[1] See Castellani, "Fungi and Fungus Diseases." Am. Med. Assoc. Chicago, 1928. Also Buchanan, "General Systemic Bacteriology." Baltimore: Williams & Wilkins Company, 1925.

specific names for cultures from different sources, of this we are sure:

Microbes having in common the characteristics ascribed to those we have called the higher bacteria, or branching bacteria, are found frequently in the soil, less often in plants and comparatively seldom in man and lower animals.

In the soil many of them produce enzymes that play an important helping part in breaking up organic matter into ammonia, carbon dioxide and water. They assist the molds and the bacteria in this decomposition, or the changing of these materials into forms to be absorbed by the higher plant roots. Many varieties of this type have been described by Waksman [1] and others as existing in the soil.

In higher plants, while some forms appear to be harmless parasites, a few varieties are known to cause destructive diseases. Thus, the different scabs on plants are due to varieties of actinomyces. These may be carried, probably only mechanically, by the aphis, that little plant louse, green, woolly, or other kinds that may grow to enormous numbers and soon cover the leaves of our delicate plant shoots and suck their juices.

In lower animals, particularly in cattle but occasionally in horses, sheep, pigs, goats and elephants, this type of microbe may cause serious diseases that may resemble tuberculosis or glanders (farcy). Animals pasturing in river valleys and other moist lowlands are more liable to contract it than those on the higher hills.

The organism which commonly attacks first the lower jaw or the tongue or the neck tissues is sup-

[1] Waksman, "Studies in the Proteolytic Enzymes of Soil Fungi and Actinomycetes." "Jour. of Bact.," November 3, 1918.

posed to gain entrance through a wound, however small, that may be made by a bearded grain or any coarse rough forage containing the spores. The disease is often called lumpy jaw because of the swelling often formed on the lower jaw. The meaning of the term lump is incorporated in the name actinomyces. It is also called wooden tongue because of the swelling and hardening of the tissues of the tongue through infiltration with inflammatory cells. There may be swellings also on the neck forming a sort of wen. All of these swellings may gradually break down in the center, forming a collection of pus or an abscess.

Stained sections of these disgusting-looking abscesses examined under the microscope may appear very beautiful—at least to the pathologist. The small yellowish or greenish-yellow granules of pinhead size seen by the naked eye scattered through the pus or in the tumor-like granulation tissue, through the lens are seen to be made up of striking-looking rosettes. These are the microbes that cause the disease. Their growing in these characteristic ray-like rosettes, combined with their causing so often a lump in their host's tissue, is the reason that Herz gave them the name actinomyces, and the reason why the disease they cause is called actinomycosis.

In the center of these older rosettes is a bunch of short threads, rods and granules. Radiating out from this center are many Indian-club or pear-shaped forms which are ends of the microbes that enlarge as they come in contact with the tissues. With the double pink and violet Gram stain the bacillar and coccal forms retain for the most part the violet stain, being Gram-

positive or nearly so (Gram-amphophile). The Indian-clubbed ends lose the violet stain by Gram's method of staining and take the pink stain so intensely that they appear glistening.

In man, alas! some of these branching forms also cause dangerous diseases; and they are among the insidious attackers. We don't know when they enter, and by the time they make their presence felt we can seldom trace their mode of entrance.

While they infect few people, they are more incriminated than earlier, particularly throughout the upper Mississippi Valley and parts of the Northwest States. At the Mayo clinic in Rochester, Minnesota, Sanford reported 157 cases in nine years (1913-22). At the Massachusetts General Hospital, sixty-three cases came under observation during the twenty years before 1922. Seven hundred cases were reported in the whole United States up to 1923.

If the person affected doesn't put up any resistance, the microbe may pass slowly through the blood vessels and become deposited in different tissues, causing a collection of tissue cells at that point which finally break down into abscesses, in which may be found the characteristic little dirty-looking yellow particles that are made up of the same kind of rayed rosettes found in the abscesses of lower animals.

If these abscesses are formed in the lungs the diagnosis may be easily made by finding the little yellow grains of rosettes in the sputum. Crushed under a cover glass and on a glass slide and then examined under the microscope, the rosettes can be seen even without staining, but staining makes them more easy to find. Some

varieties of these microbes do not make rosettes in tissues, and none of them form these striking growths readily in cultures.

Whether the microbe may be normally present in the mouth for any time ready and able to invade the blood stream and tissues whenever a wound is made and the person's resistance allows it, or whether it is only taken in with the food from time to time, is a moot question, as is also the question of the part played by at least some of these microbes as secondary invaders.

That they may be in carious teeth has been clearly demonstrated by the fact that the injection of the contents of such a tooth into the peritoneal cavity of a guinea-pig has been followed by the development of actinomycosis in the animal. But just what part they have, if any, in bringing about the caries of the tooth is not clear.

Castellani has described a number of cases of chronic tonsillitis, occurring commonly in the tropics, that he considers are due to this type of microbe. He says that the tonsillar crypts become plugged with a yellowish-white mass consisting of a number of different kinds of microbes, chief among them being these slender branching forms. The whole mass may emit an offensive odor, which is usually the cause of the patient consulting the doctor. That the germs collected in these crypts are not of the usual invading type is shown by the fact that they simply stay in the crypts, accompanied by only slight enlargement of the tonsils, and that they are easily removed and the condition cleared up by proper treatment.

What such forms might do in the occasional non-resistant case we don't know. We do know, however,

that a few abscesses of the brain have been found to be due to this type of microbe, also a few cases in which the linings of the brain are the parts they attack.

Only eighteen cases of this kind of meningitis have been reported in the whole world, according to Dr. Neal. But all of these cases died. The majority of these cases had been suffering from middle ear infection, so presumably the branching organisms were in the pus of the ear and reached the meninges from this source, but no definite evidence was given that this was the case. In these brain cases the course of the apparent disease may be very short.

One of the cases seen by Dr. Neal, the one in consultation with Dr. Elmendorf, may be cited as an illustration of how such infections may finally give evidence of their presence in the brain and what a rapid course they may run. No point of entry could be determined. The boy, a child of twelve years, had seemed well for some time before. It is true he had been a "blue" baby and he had always been a delicate child, but he had been well cared for and during the past half dozen years had seemed reasonably healthy and had had a comfortable life.

One day after he had returned from a trip to Virginia he complained of a headache which lasted only a short time. During the next few days he complained again several times for a little while at a time of having a pain in his head. His parents thought he was merely a little upset by his trip, but he continued to complain off and on of a head pain. So his parents called in the family physician. He couldn't find any cause for the headache. During the next few days the child developed a low irregular fever and he vomited

once. Then suddenly eleven days after he first complained of his head he became much worse. He went into convulsion after convulsion. At this time the chief of the division caring for the meningitis diseases of the Health Department, Dr. Neal, was called in consultation.

Of course, by this time there was no question but that the child had meningitis. So the laboratory doctor took fluid from the spinal canal and gave the child serum. The spinal fluid was taken to the laboratory and submitted to the usual rigid examination. Then they put some of it under the microscope to look for the microbe that usually causes meningitis, the meningococcus. But they didn't find the characteristic Gram-negative diplococci. Instead they found innumerable minute Gram-positive and Gram-negative cocci, with occasional bacillar forms. What was it? "That," said the chief examiner, to whom the specimen was referred, "looks very like one of those higher bacteria that will show branches if you put it in a right environment. Wait until you see what you get from the cultures you have made."

When they examined the cultures after letting them grow overnight at blood heat in the incubator they found no growth on the surface of the culture medium that always shows a growth of the meningococcus, when present. Then they looked at the tubes of the broth and serum medium in which they always place a little of the material to be examined and pour over the top some liquid vaseline in order to give those microbes that cannot grow on surface in the presence of air a chance to grow. And there they found a delicate cloud at the bottom of the tube of this medium.

Examining a drop under the microscope, they found that the cloud was caused by a growth of a delicate thread-like microbe with an occasional branch and many coccus forms. On further study, giving it the sugars this kind of microbe particularly likes, it indeed proved to be a branching microbe—an actinomyces. The child died in three days from the time it began to have convulsions.

The microbe obtained from this case was, as we said, an anaërobic variety of the branching germs. From other cases, especially from lung infections, cultures have been obtained of a microbe very similar to these anaërobic forms, except that they will grow in the air—that is, they are aërobic. These aërobic cultures are more apt to form the spore-like bodies that some people think should be a basis for grouping them under a separate name.

Varieties of these branching microbes have been found in skin diseases. In one of these conditions a purulent inflammation of the foot, called mycetoma or Madura foot, occurring in warm countries, a variety of actinomyces, is accepted as the cause of the disease as it occurs in India (Vincent), and another variety in a similar condition occurring in the Philippines (Musgrave and Clegg).

In certain cases of rat bite fever, Blake, Minsnerkoff and others have found actinomyces-like organisms in the blood. In some cases of pyelitis, or inflammation of the bladder, an organism of this type has been present, but just how much harm it is capable of doing is not determined.

In a certain febrile disease of cattle a thread-like bacillus called actinobacillus has been found. It is con-

sidered a near relative of the actinomycetes. In an erysipelas-like disease in swine an actinomyces-like microbe has been found called erysipelothrix. It is considered the cause of the disease.

In all cases where an actinomyces infection is suspected potassium iodide should be given in large doses, since in some cases it has had a remarkably quick curative action, in both man and beast.

If given to milch cows, it causes the milk to dry up. In market animals, when the condition is local, the diseased parts may be cut out and the rest of the meat put on the market.

## CHAPTER XVII

## YEASTS AND MOLDS

Alcohol producers—Bread raisers—Cheese flavorers and ripeners—Decomposers of organic matter—Infrequent producers of disease.

It is little wonder that much was learned about molds and yeasts long before knowledge of bacteria became general. They are so much bigger than bacteria and grow in such conspicuous ways, from mushrooms to ergot, that they thrust themselves upon one's notice. An ordinary hand lens magnifies enough to show, in some varieties, the beautiful fruiting branches waving in the air, and a little higher magnification reveals the interlacing filaments or threads (myceleum) of the molds and the budding cells of the yeasts.

Moreover, they are found everywhere. Their minute fruits, often containing many seeds or spores, have so little weight that they may be carried far by even a breath of air. They are so resistant to unfavorable surroundings, such as heat, cold, lack of moisture, and so on, that they are not easily destroyed and they may remain quiescent for an indefinite time. Then, with any encouragement, the spores sprout and begin to grow rapidly.

Such varieties need only the slightest amount of moisture to enable them to start growing, and they find food in the most unlikely places. Once finding it,

if they be undisturbed long enough they grow luxuriantly.

Thus they grow on old leather shoes, covering them with irregular green patches that might be considered beautiful elsewhere. They invade our rugs, stuffed furniture and bedding, developing an unpleasant musty smell during damp summer months if these household furnishings are not frequently and thoroughly beaten and exposed to sunny air.

They are very fond of sugar, so if given half a chance, they cover our jellies and jams. Some varieties grow even in raw sugar, where they may cause considerable damages in commerce.

They may grow upon and in all kinds of exposed food, thus spoiling it at least for our taste. So, while the vast majority of them give no evidence of attacking man's body, they manage to make themselves very disagreeable or at least apparently useless: except—and there is a big except—some varieties were early found to produce effects that are both agreeable and useful to man, and a few varieties, alas! have shown themselves able to attack him and cause serious disease. In fact, a mold was the very first microbe that was accused of causing a disease. In the little yellow cups of favus, a disease of the scalp, a microbe was found by the German Schönlein as early as 1839, and was thought by him to be the cause of this skin disease, though other observers at that time thought that this was a peculiar hypothesis.

In the next few decades molds were found in several other obvious skin diseases, but interest in them as human disease agents died down when the more spectacular facts in regard to bacteria and disease were

brought to light. In later years other varieties of this type of microbe have been convicted of causing dangerous diseases in man, beast and plants.

In the meantime, the interest in those forms that were found to make man's life seem more enjoyable and useful to him continued to increase, until now we have great industries whose products are aided by the employment of these agencies.

So there is no question about the important rôle played by the yeasts and molds in the life of man, in both helping and hindering his—shall we call it?—progress.

In the first place, let us consider those useful varieties that have helped form great industries. Chief among them are certain yeasts. Doubt, nay, absolute conviction, is found in the minds of many as to the value to man of the principal use to which these yeasts have been put. For they are the great agents in producing alcohol.

What a drama in the world's history of microbes was the discovery that yeasts make alcohol out of sugar; that living things cause fermentation; that yeasts ferment grapes and other fruit into wine which may be changed into brandy; that yeasts make beer from barley, corn whisky (moonshine whisky) from corn; whisky "the breath of life," from many different grains; that they make alcohol from beets, from molasses, from any material containing sugar, and allowing them to grow.

What years of brilliant experiments and telling demonstrations were made by Pasteur and others in their efforts to show the world that the early discoverers, Latour, Kutzing and Schwann, were right, and that Liebig and others were wrong; to show that fermentation

really was caused by these little budding microbes and that alcohol was produced through their agency.

Since that time much has been learned in regard to the best yeasts to use and the best methods of growing them in order to produce certain results in the making of alcohol in general, and in the manufacturing of wine, beer, brandy and other alcoholic beverages and medicines, in particular. Metaphoric and rhetoric use might be made by the "drys" of the fact that the alcohol produced by the yeast finally kills it. At any rate, real use is made of this fact by the manufacturers of spirituous liquors in their clearing of the products.

What clever experiments were made also by Pasteur to show how "wild yeasts" and certain other microbes interfere with the making of good alcoholic liquors. Much research has been carried on as well concerning the mechanism of the reactions in the production of alcohol.

Certain yeasts also play a part in another famous industry. This is due to a further power they have, that of forming a gas in their growth. This rôle, of course, is played in the making of bread. Every one knows yeast cakes and the great industry that has arisen from their power to "raise bread," to make it porous and light. And not only do cooks add them to their ingredients for making bread, but many people have eaten yeast cakes directly, with the hope of curing certain gastro-intestinal and skin disorders, such as constipation and acne, or of adding more vitamins to their diet. The hope for yeast being of use in these diseased conditions has not been realized. The yeast seems to have no inhibiting effect on the putrefactive bacteria in the intestinal canal, and, while constipation

is relieved in some cases, in others diarrhea develops, so some observers think that yeast eaten in quantity, while usually harmless, might sometimes be harmful.

One of the by-products in the manufacture of alcohol from yeast is glycerin. By the usual method only a little is produced; but during the World War, when glycerin was wanted and the supply was low, several people made attempts to get an increase from this source. Finally, two Germans found that if fermentation was carried on in an alkaline solution much more glycerin was produced.

Other studies of yeasts being carried on at present may yield still more interesting results.

A variety of the yeast group is an active agent in the ripening of Swiss, Belgian and other cheeses. In the making of some cheeses certain molds are used.

In Roquefort cheese the green color is due to the common mold Penicillium glaucum. This is considered such an improvement to the flavor of the cheese that it is the practice to sow it purposely in the cheese mass. This is done by allowing bread to become covered with a rich growth of mold, then drying and grinding it and spreading the resulting powder between the separate layers of the sliced curd. In several other cheeses also molds have been used to improve their quality.

The molds have been used by humans in a number of other ways. Thus, certain molds have an active power in breaking down or fermenting various substances besides carbohydrates by their special enzymes, so that these substances are in form to be fermented by yeast cells. In Japan, in the production of spirituous liquors like that Japanese alcoholic drink known as saké, rice is acted upon first by a mold of the

Aspergillus group, and then by a yeast. Another variety is used for making a special preparation from the bean. A number of these mold enzymes have been patented in Japan and placed on the market.

Several organic acids are manufactured through the agency of mold fermentations, notably gallic acid from tannin and citric acid from granulated sugar. Thom [1] in his interesting sketch of one of the most representative of the molds, the Aspergillus, describes more fully the points given above and many others.

He calls attention to an interesting habit of the fruits of some of these molds. When growing on a medium that is neutral they are purplish in color. If an alkali is added to the medium, they turn blue; if an acid is added, they turn red. They might thus be used as an indicator in determining whether an organism produces acid or alkali in its growth.

Waksman [2] sums up our knowledge of the important part played by molds in the decomposition of organic matter in the soil. Different molds attack different substances. Thus mucor molds attack the proteins and the hemicelluloses, but not the true celluloses, or the tannins or certain others, while Aspergillus, Penicillium and several other varieties break these up into simpler constituents. The decomposition of these substances is helped largely by certain bacteria, as we mentioned in Chapter VII.

Now we come to the varieties of the molds and the yeasts that exert a harmful action on man. The majority of those that attack man invade the skin and

[1] Thom, "The Aspergilli," in "The Newer Knowledge of Bacteriology," etc., p. 437. University of Chicago Press, 1928.
[2] Waksman, *ibid.*, p. 315.

hairs only. But a few penetrate deeper, and these may be so insidious and persistent in their growth through the tissues that, if not discovered and treated in time, they may finally kill their victim. The more superficial growers may only cause baldness, the loss of finger nails, disagreeable itching, spotting and pimpling of the skin, and so on.

Not only are there those that harm man thus directly, but there are many varieties that cause indirect harm to man in instigating wide-spread plant epidemics. Potatoes, oats, wheat, cotton, many fruits —all may be attacked. Whole coffee plantations have comparatively suddenly disappeared in Ceylon, says Castellani, through epidemics caused by fungus.

Varieties of microbes all among the molds that cause certain skin disease in man were the first to be discovered in those attacking the hairs and the earliest to attract attention. These occur with greatest frequency in the tropics and usually among people who are careless in habits of hygiene. But northern countries are by no means free from them.

Favus, in which little yellow cups are formed about hairs; the various kinds of ringworm, that danger from unclean barber shops; the mange, that may make our dogs and other animals unsightly; tinea flava, causing a loss of pigment in the skin, making light spots on the dark skin of southern natives, which are considered beauty spots by them—all of these and a number of other skin diseases are due to molds. Most of these were seen for the first time about the middle of the last century. While they were accused by their discoverers of being the cause of the conditions in which they were found, by many observers they were not believed to

be the cause. As time went on, with the help of many investigators and of experiments on volunteers it was finally proved that certain molds caused these diseases.

Then it was found that yeasts could cause pustules and ulcers in the skin, that these may slowly increase in numbers, and that the yeast may finally travel through the whole body, producing abscesses wherever they may lodge and finally causing death.

The first papules and pustules formed by the yeast are not painful, so in the beginning they may not be taken care of properly, and then by the time they are recognized as harmful it may be too late for any good effect from the potassium iodide which we usually give in these cases.

The question of the relationships of the various strains of yeasts isolated from human disease is still far from being settled, but this does not interfere with the practical point of diagnosis, nor with the knowing how to treat those who are infected.

One of the first invaders of human beings among the yeasts was discovered by Busse in 1894. The yeast appeared first in an abscess of the tibia, then gradually it infected lymph glands, lungs, kidney and spleen. It took over a year to kill its victim. In the majority of the generalized infections ending fatally the lung has been the chief site of growth of this kind of microbe.

It often looks something like a protozoan growing in the tissues, and was first said to be one of the animal microbes. Then Orphuls showed that it could grow and put out mycelial threads like a yeast mold.

The brain of humans too is not exempt from invasion by this type of microbe. About the same number of people have been reported as having a fatal

brain infection of yeasts as those killed through brain infection by actinomyces. The course of the disease is generally longer than in actinomyces infections. Here is a sketch of a case Dr. Neal saw in consultation with Dr. Shapiro:

In New York, a boy sixteen years old had always been reasonably well until February, 1923, when he complained of left frontal headache, of weakness and of sleepiness, which persisted for about one week. At this time he did not appear to be suffering from any acute or chronic disease. His mentality was normal, and physical examination especially of the nervous system, eyes, ears, nose and tissues was negative.

Suspecting encephalitis, he was kept at home another week; at the end of that time he felt so well that he returned to his private school.

He was well until May 15, when, after eating a large quantity of chocolate cake and pie, he suddenly developed a similar left frontal headache with vomiting, weakness and dizziness, which continued off and on. Not responding to systematic treatment, he was sent to a sanatorium on May 23. Physical examination at this time was negative, except for a loss of fifteen pounds (6.8 kg.) in weight since February. Mentally, he was normal until the evening of May 25, when he suddenly became delirious. This lasted but a few hours. Five days later the patient complained for the first time of pain in the back of the head and neck, slight disturbance of vision and photophobia. From then on evidence rapidly developed that we were dealing with an acute infection of the central nervous system, particularly of the meninges.

Lumbar puncture was performed for the first time

on May 29. The fluid was found to be under very high tension and slightly hazy in appearance. There was no growth in the culture of the fluid the first eighteen hours, but distinct yeast colonies were apparent on the following morning, May 31, thereby establishing the diagnosis of a yeast infection of the central nervous system. Lumbar punctures, which seemed to offer him some relief, were subsequently made daily until near the end, and the torula organism was recovered from the fluid by culture and observed in smears. He became gradually worse and died about eight months after first being seen.

The iodine treatment as well as various other remedies tried seemed to have no effect.

These microbes certainly have a way of keeping right on growing in the exceptional case once they get started.

One of the definite molds called Sporothrix has been found in quite a number of skin diseases and in a smaller number of general infections in man.

In this infection if given in time potassium iodide may be said to be a specific cure.

## CHAPTER XVIII

## ANIMAL MICROBES OR PROTOZOA

The amœba as a fighter—The misnomer malaria—Protozoa in mosquitos and man, in ticks and Texas fever—Sleeping sickness and the boring animal—The animal causing dum-dum disease.

Microscopic animals are neither as wide-spread nor as numerous as are microscopic plants; moreover, those animal germs that are definitely harmful to man are all parasites. There are no free living—saprozoic—varieties known among them. None of the varieties that cause disease in man have been shown to be able to multiply outside of an animal under natural conditions. Of course, those that form cysts may exist for a variable time in a quiescent state outside of their animal hosts. The majority of those that attack man are met with commonly in tropical countries.

Neither are the protozoa easily transmitted from man to man. Indeed, most of them are transmitted chiefly if not only through a second host, commonly a biting insect, in which they must usually undergo a period of development before they can infect man. So these forms do not cause definite epidemics. Rather they are endemic in certain localities.

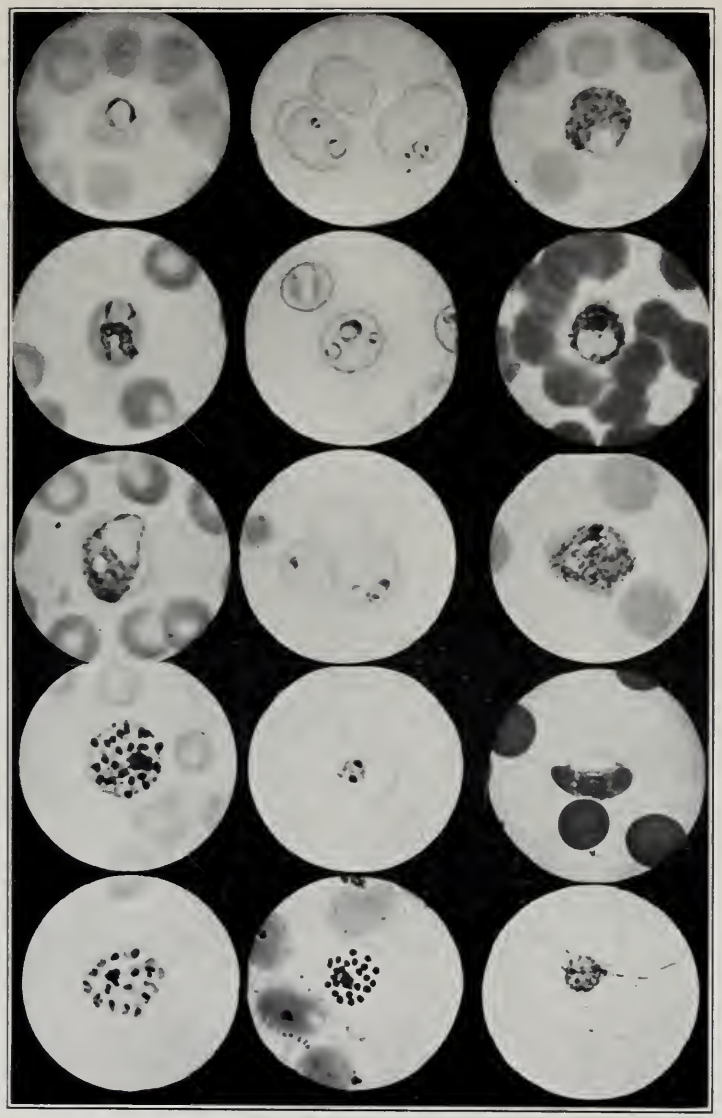
While many of these animal microbes are not directly harmful to man, not many, if any, are known to be helpful to him. It is true that some of the protozoa in

the soil which may be teeming with flagellates and amœbas may help decompose organic matter and prepare it for plant growth. But on the other hand, some observers claim that the soil protozoa eat up the bacteria that are the most helpful aids in the life cycle of plants and animals. Thus they really make the soil less fertile. As evidence in favor of this idea the fact is cited that partial sterilization of soils that kills off most protozoa results in greater fertility for the higher plants.

The first protozoan introduced as a disease producer in man was an amœba, that microbe favored as the type of our beginning. But what its relationship was and is to others of the amœba family we cannot say. For, alas! here again the descriptions of the form first seen, in dysentery, by Lösch, away back in 1875, and the varied attributes claimed for varieties introduced since then, leave one in doubt as to whether or not this first one is an ancestor or other relative of any of the later ones.

How limited is our field of vision and how quick we are to draw deductions from a few one-sided observations! All research tells the same story. Again we must say that it is only the exceptional one that sees clearly, that checks or controls his observations at every step and that is slow to draw deductions. The story of the introduction of the pathogenic and the saprogenic amœbas is a striking illustration of the truth of these laments.

We are still not sure how many varieties of amœbas cause disease, but we know, from the fine work of Councilman and Lefleur and others, that one at least, common in the tropics, may cause a dysentery characterized



Stages in the life of different types of the malarial protozoa growing on and in red blood cells. Enlarged 1000 diameters. (From Kolle & Wassermann.)

Protozoa called Leishman-Donovan bodies that were thought to be sporozoa until they were grown in pure cultures when they developed flagellated forms like those in the illustration, which placed them among the flagellates. (After Nicolle.)

by discharges of blood and mucus which may be followed by abscesses of liver, lungs and brain. This pathogenic form now goes by the name Endamœba histolytica, but it has many synonyms and its history is all mixed up with that of its supposedly harmless near relative, Endamœba coli. The nucleus is said to be so different in these different forms that they can easily be recognized by its characteristic appearance. And the number of nuclei in the cysts is quoted as a differential point. But it is disheartening to the lover of the immutable to think how these two factors may vary.

We were able to show that several varieties of amœbas obtained from the feces of different animals, including man, all quoted as "free living" forms by experts—that is, forms that grow only outside of the animal body—are able to grow in pure cultures, feeding only on fresh animal tissue placed in artificial media in test tubes or Petri dishes. Such culture forms, growing vigorously and continuously without bacteria on successive transplants of this tissue medium, present appearances varying enormously in each strain with the temperature at which they are allowed to grow, as well as with other factors.

Fortunately, though we may not know what specific name to call an amœba when we see it in the feces, we know what to do to prevent amœbic dysentery. We must keep food and drink from fecal contamination, which means personally fly swatting and clean hands. We must protect the water supply. Easy to say, but how difficult to do! At least suspicious water can be boiled and vegetables and fruits cooked, or washed thoroughly and immersed in boiling water for ten seconds.

Then we have a cure, though with exceptions again. Emetin in various forms and combinations has benefited and even seemed to cure a number of cases.

A few years ago great excitement prevailed among the dental profession when two investigators announced that pyorrhœa, that dread of lovers of good teeth, was due to an amœba. We soon showed that the poor amœba wasn't to blame in this case, at least primarily, if at all, though large numbers were found in decayed and dirty teeth.

Some investigators now believe that certain amœbas may get into our joints and bones and cause symptoms of arthritis, and this time their belief may prove to be the truth, though we have yet no clear-cut evidence.

The next protozoan shown to be pathogenic for man is the one causing that misnomer malaria (bad air). What a discovery this was! The Italian Laveran maintained in spite of much opposition and ridicule that the minute amœboid forms he had found in the red blood cells of man during attacks of malaria were living microbes and the cause of the disease. And after his critical colleagues really studied the disputed forms, they had to agree with him. He called his organism Plasmodium malariæ. Then they found that there were three types of these protozoa that cause malaria. One taking two days to develop is called the tertian type, because the dreaded chill and fever come every third day. The second form, taking three days to develop, is called the quartan type, because the chill and fever come every fourth day. Then there is the worst form of all, or it may be a mixture of forms, the so-called Estivo-autumnal or pernicious type, in which the chill and fever may occur every day with irregular re-

missions, or the infection may be so intense that the victim may be struck down with delirium followed by coma and death in a very short time. This form may affect some of its victims in other bad ways, producing chronic disease and recurrences.

All of these bad forms still are active in the tropics when the proper means of controlling them cannot be carried out, but with the advance of public health control the malarial parasites have practically disappeared from many parts of the world where they once held sway.

Notwithstanding the fact that the wonderful empyric discovery in the eighteenth century of the telling effects of quinine on the disease caused by these parasites was made long before the parasites were brought into the limelight, still, as late as fifty years ago the disease was common throughout much of the world as well as in the tropics. What years of misery it caused to many a life, young and old! What fantastic ideas there were concerning its cause! Malaria meant air from swamps, from damp places, from excavating ground, air that descended upon one at night so that one carried an umbrella to ward off this falling evil.

And then came the stunning demonstration by Manson and others of the fact, surmised long before by the few as usual but not considered by the many, that mosquitos carry the malaria germs; that it was the mosquito that rose from damp swampy places, that descended upon us by night, that bit us freely in those days when we had no fear of it except its disagreeable effects; it was this despised mosquito who was shown to be one of the mighty enemies that man must fight.

And fight it he did with oil, draining away waste waters, with minnows and other enemies of the larvæ, with screening, and with treating and protecting human cases. Now we in the Middle and Northern States of the United States have practically no malaria. The special mosquito carriers, the anopheles, are seldom seen here at a glance.

In the meantime, mosquitos were studied all over the world, and many curious facts were learned in regard to the different varieties. The habits and distribution of the carriers of the malarial microbes became very well known and many mysteries in regard to malaria and the mosquito were cleared up.

Some of the differences between the "malarial mosquitos" (anopheles) and the more common harmless varieties (culex) are shown in the illustration.

The first step in working out the wonderful cycle of life of the malarial germ was made by MacCallum's exciting discovery that there were sexual forms of these organisms. Then Grassi and others worked out the complete life cycle, showing that after infected blood was imbibed by the mosquito the malarial male and female germs fuse, then grow into a large body full of little flagellated forms; these pass to the salivary gland of the mosquito and from there they reach the blood of a human being when the mosquito bites him. When these small forms reach the blood cells of the bitten human they round out into amœboid forms, grow quite big and divide into a number of minute amœboid forms ready to infect new blood cells, or be imbibed by another mosquito.

An organism that is considered a distant relative of the malarial microbes was found in 1888 by Babes

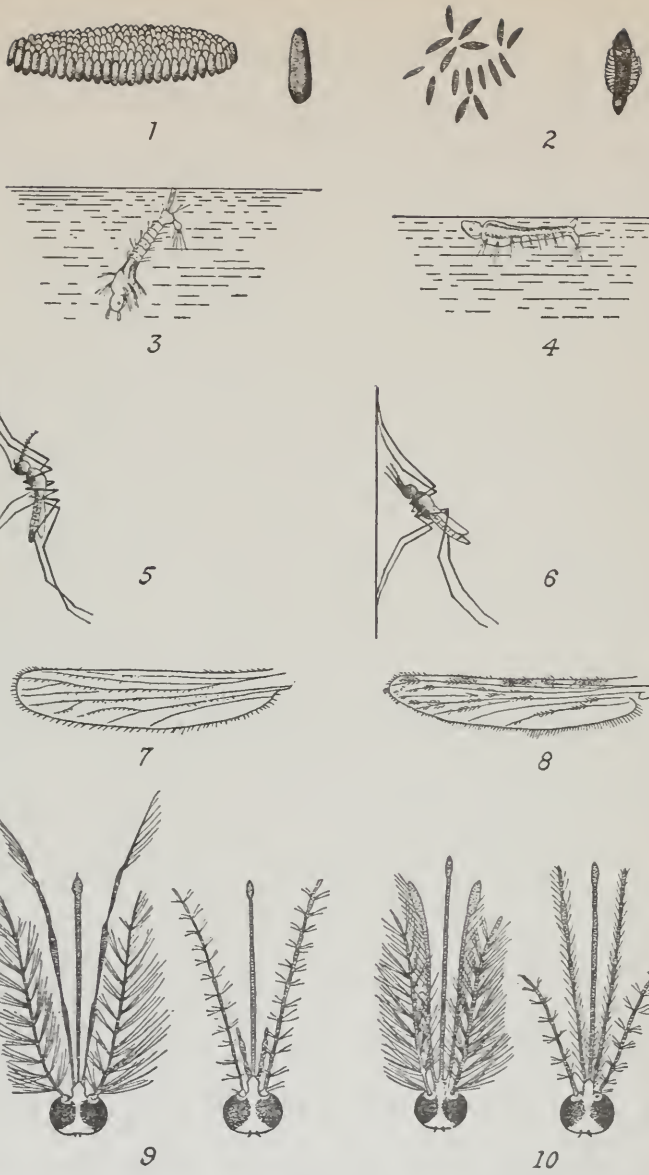



*Fig. 10*

CHIEF COMPARATIVE CHARACTERISTICS OF CULEX AND ANOPHELES. (FROM KOLLE AND HETSCH.) EGG OF CULEX, 1, LAID TOGETHER IN "SMALL BOAT," THOSE OF ANOPHELES, 2, SEPARATE AND ROUNDED. LARVA OF *C.*, 3, HANGS NEARLY AT RIGHT ANGLES TO WATER SURFACE, THOSE OF *A.*, 4, ARE PARALLEL TO SURFACE. BODY OF *C.*, 5, WHEN RESTING IS HELD PARALLEL TO WALL IN A CURVED POSITION, THAT OF *A.*, 6, STANDS AT AN ANGLE OF ABOUT 45° AND IS STRAIGHT; WINGS OF *C.*, 7, ARE GENERALLY NOT SPOTTED, THOSE OF *A.*, 8, ARE SPOTTED. IN *C.* THE PALPÆ, 9, OF THE FEMALE ARE VERY SHORT, OF THE MALE ARE LONGER THAN THE PROBOSCIS; IN *A.*, 10, THE PROBOSCIS OF BOTH SEXES ARE ABOUT OF EQUAL LENGTH.

in Rumanian cattle and independently by Theobald Smith and Kilborne in cattle throughout Texas, suffering from Texas fever. This microbe is now called Babesia bigemina.

While this form has not been found to attack man, it affects him indirectly, and an important fact in its life history made its discovery of still greater practical worth to us. Smith and Kilborne showed in a masterly piece of research work that the infection was borne by a tick and transmitted to a new bovine by the offspring of this tick. They demonstrated this some time before Manson showed that the mosquitos carried the malarial germ, so they were the first to direct our attention to the important fact that lower animals may be hosts or carriers of some of our microbe enemies.

Smith showed that if cattle were kept from infected fields for two years and non-susceptible animals (horses and mules) were allowed to feed there the bad ticks might disappear. In the meantime, they showed that ticks on animals might be killed by allowing the animal to pass through an oil bath.

The serum from animals that have recovered from Texas fever has been tried out for its preventive qualities, but it has been found to be of little use.

Minute microbes like these Babesia have been found in cattle in other parts of the world. A similar germ has been found also in dogs in certain parts of the world. This last variety has been studied exhaustively by Nuttall and Graham Smith, but they didn't succeed in getting it to grow. Another variety has been found in horses and still another variety in sheep. Each variety seems to be able to infect only the one kind

of animal, and each to be transmitted by one kind of a tick.

There has been no more surprising discovery of the cause of disease in man than that in the disease known as tropical sleeping sickness. This is quite a different disease from the one also known commonly throughout a larger part of the world as sleeping sickness, but whose real name is Encephalitis lethargica. This one will be spoken of in the next chapter.

For years in the tropics many natives had been attacked by gradually increasing weakness and somnolence, finally ending in death. The condition was rightly called the sleeping sickness.

Then, just at the time Smith made his discovery of the cause of Texas fever, Evans and a little later Linguard found in the blood of horses in East India, suffering from a wasting disease called surra, some actively motile peculiar flagellates like those found earlier by Lewis and others in the blood of about one wild rat in a hundred throughout the world, and like those found so long before (1843) by Gruby in the blood of a frog. Gruby had named his find trypanosoma, which means a boring animal, because of the appearance of a bore given by the fluted frill winding about its body.

Neither the rat trypanosome nor the frog trypanosome seemed to make its host sick. But in quick succession different investigators found in animals in different parts of the country, each suffering from a disease like surra, a similar boring-looking microbe, and they proved by inoculation experiments that the microbe really causes the disease. In the midst of these

discoveries Bruce found that a special biting fly, called tse-tse fly, is instrumental in conveying the trypanosome to another host.

No one had any idea that man was susceptible to this kind of organisms.

Then one day Nepvieu (1898), while examining the blood of some drooping natives in Africa to see if he could find any malarial organisms there, saw the blood cells being pushed about violently by some squirming object. He watched it until he became convinced it was one of those boring-looking trypanosomes. He found a few more in several other cases; then Dutton and Todd and others examined many of the natives and found a few more. Finally, Castellani found trypanosomes in the centrifugalized spinal fluid of twenty out of thirty-four cases of definite sleeping sickness. His work has since been fully corroborated. White visitors in the country were also found to be susceptible. Various species of biting bugs have been shown to be able to transmit these microbes. The reason these striking-looking fluted microbes were not seen before is chiefly because so few are in the blood at any one time.

Novy and MacNeal in America were the first ones to obtain a pure culture of this kind of microbe. They made a soft medium of nutrient agar and rabbit's blood and injected into it some of the blood from an infected case. These blood parasites grew in the water of condensation, and continued to grow in successive transplants in this artificial culture medium.

All of the harmful trypanosomes first discovered were found chiefly in animals and natives of certain parts of Africa, where they may cause terrible infections. Later a variety was found to be causing much loss of

life among cattle and horses in the Philippines. Then Chargas found that a disease occurring chiefly among children in Brazil is caused by a peculiar kind of trypanosome that passes part of its life cycle in certain of the tissue cells of its host, where it looks very like the Leishman-Donovan bodies to be next described.

The symptoms caused by these trypanosomes are similar in all of these conditions known collectively as trypanosomiasis, or sleeping sickness. The victims have irregular low fever with gradually developing emaciation, tremors, incoördination, somnolence, coma and then death; always the end was death. Now, with the application of the wonderful drugs that have been discovered, the treatment of these conditions seems to promise great results. Atoxyl, combinations of antimony, and the latest and most hopeful drugs, a new combination of arsenic synthesized at the Rockefeller Institute, and the Bayer 205, a patented German drug, whose composition is not made public, seem to be effective as cures in the first stages of the disease in all of the forms except that occurring in Brazil, where no drug seems yet to have any curative effect.

Another peculiar insidious, usually highly fatal, disease of the tropics called dum-dum fever, or kala-azar, used to be considered a malarial cachexia. Then, in 1903, Leishman and Donovan independently found some queer looking bodies within certain of the spleen cells of persons sick with this fever. Their discoverers judged from the appearance and position of these bodies that they were animal parasites and probably the cause of the disease. Their judgment was found to be correct. The bodies were known first as the Leishman-Donovan bodies. Later, when it was proved

that they are really protozoa, they were given the same name but in a scientific form, Leishmania donovani.

That same year a native of Delhi arrived in this country at Boston with what is known as a Delhi boil or Oriental sore on his face. Wright examined this and found it loaded with the same kind of bodies. Since each of these microbes produces a disease so different from the other, each is called by a different species name. Wright's bodies are known as Leishmania tropica.

The sand-fly is probably the chief culprit that transmits these enemies.

Several interesting facts were brought out in attempting to cultivate these microbes. No one at first could get them to grow. Then Rogers, instead of putting at blood heat all of his tubes containing bits of infected tissue in Novy and MacNeal's culture medium, left some at room temperature. When he examined these he found evidences of growth. Some were dividing and some elongating. Not only that, but some of the elongated forms began to wiggle, and behold! there was a flagellum growing out from one end. So they were flagellates, instead of being exclusively cell parasites or sporozoa, as was first thought.

A combination of antimony has been found to be almost a sure cure if given early enough in cases infected with this type of microbe.

A number of protozoa, together with many of the bacteria, find the intestinal canal of man and lower animals an ideal place for living. Representatives of all four of the large groups of protozoa may be found there. Six kinds of amœbas, seven of flagellates, one

of sporozoa (coccidium) and one of the ciliates have been reported as inhabiting man's intestinal canal from time to time.[1] Very little harm is done their hosts, except by the amœbas.

But since some of these may be at least the cause of disagreeable diarrheas and might at most develop more dangerous qualities, it is well to be on one's guard against carriers and infected food, particularly in those countries where these infections are more common.

[1] See Hegner on Intestinal Protozoa of Man, p. 660, in "Newer Knowledge of Bacteriology and Immunity," ed. by Jordan and Falk. University of Chicago Press, 1928.

## CHAPTER XIX

## UNKNOWN MICROBES

### FILTERABLE VIRUSES

Ultra-microbes, the smallest of all, the cause of yellow fever, rabies, smallpox and a number of other diseases.

HERE are mysteries for you! A whole group of diseases exist due each to an infectious principle—a virus that has not yet been identified as a microbe. As many of these viruses in diluted suspensions pass more or less readily through very fine porcelain or stone filters, they are called filterable viruses. These filtrates may look like clear limpid water and yet be very infectious; that is, they may be capable of reproducing the disease when injected into susceptible animals. Examined under the highest power microscope, no significant formed particles can be seen. The filtrate, therefore, contains either ultramicroscopic germs or minute microbes with staining powers and refraction so faint as to be non-demonstrable by known methods. Some idea of the immense numbers of investigations made by research workers all over the world on diseases due to such viruses may be obtained by reading the symposium on this subject edited by Rivers.[1]

A list of sixty or more diseases is given by Rivers that have been placed by different observers in this filterable group. Many of these are diseases of plants

[1] "Filterable Viruses." Ed. by Thomas M. Rivers. Williams & Wilkins Company, 1928.

or of lower animals alone, so they may only affect man indirectly. Fifteen of them have been reported as infectious for man, most of them lightly and infrequently, a few severely and often. A number of these affect both man and lower animals.

The process of filtration has played such an important part in the study of these viruses that we should know something about the kinds of filters used and the way to use them. The process seems very simple, but in using the fine filters, especially, the filtration is much more complex than one would think.

The material composing the filter, the method of manufacturing it, its thickness, the size of the pores, the time filtration is continued, the pressure used, the age of the filter, the preparation of the material to be filtered, tests of filters and of filtrates, all are important factors to be taken into account in the deductions we may draw from the results we obtain through the process of filtration of these ultramicroscopic viruses.

As far back as 1884 Chamberlain, working at the Pasteur Institute in Paris, showed that when he made hollow candles of unglazed porcelain (finely ground kaolin or hydrous aluminum silicate plus quartz sand or silica), closed at one end, the filter would retain the bacteria of fluid cultures, but would allow certain of their toxic products to pass through. It was found later that porcelain filters with different-sized pores could be made, the finest allowing no known microbes to pass, the next larger allowing the smallest bacteria to pass, and the largest allowing all the known bacteria to pass. Similar filters were made in different parts of the world.

Then filters were made of diatomaceous or fossil earth, now known as stone or Berkefield filters. These were also made of different grades, distinguished by letters N meaning normal, V coarse, and W fine or dense. A number of firms have made similar filters with different names, and of various sizes and thickness.

If a ground cross-section of any of these filters is examined under the microscope it is seen to be made up of granules surrounded by capillary spaces connected irregularly by larger spaces or lacunæ. The larger spaces are more numerous in the coarser types.

Other filters are of asbestos disks devised by Seitz. Then there are plaster of Paris filters and finally the so-called ultra-filters made of collodion on a porous support used chiefly for the separation of colloids.

When we once get our filters made, the first thing we do is to wash them by forcing distilled water through them. This rids them of any loose foreign matter. A preliminary air test may then be done which, according to the rate and size of bubbles arising under a certain pressure, indicates either a gross defect or the size of the pores.

Then the filters must be sterilized. This is usually done by exposure to moist heat in an Arnold sterilizer for several hours. Autoclave sterilization must be used with care, since there is danger of injuring the filter by too great a heat.

After this the filter chosen for use is attached to a pressure pump and is tested for its ability to hold back certain known organisms of different sizes. It is then ready for the material to be filtered.

This material is prepared in special ways according to its character. If it contains any coarse particles,

these should be removed by centrifuging or by a preliminary filtration through paper pulp or other coarse filter. If it is thick, it must be greatly diluted. Its reaction to hydrogen ion concentration must be determined.

Now comes the question of the effect of the filter on the material being filtered. First there is the probability of some of the material sticking to the sides of the capillaries or of the filter walls. This adsorption, as it is called, varies with the kind of material filtered and the kind of material making up the filters. The electric reaction of these materials (whether they are electropositive or electronegative) makes a marked difference in the results, since porcelain, fossiliferous and asbestos filters are negatively charged and plaster of Paris filters are positively charged. Basic or alkaline solutions are adsorbed by the walls of the first type of filter, while acid solutions are not; the contrary is true for the second type. But only the first part of the fluid that passes through is adsorbed. When the walls of the filter are covered with a film of the adsorbed portion the fluid then comes through without further adsorption.

To understand fully the mechanism of these reactions one should know electro-physics. Practically, it means that because of adsorption of the filtering material by the pores of the filter we may not have the same results in the first few centimeters of any filtrate that we get with later amounts filtered.

The thickness of the walls of the filter of course also influences the first filtrates coming through, as do also the strength of the dilution and the kind of diluent used in preparing the material to be filtered. The

amount of deposit from the unfiltered material on the surface of the filter also influences the rate and character of the flow through.

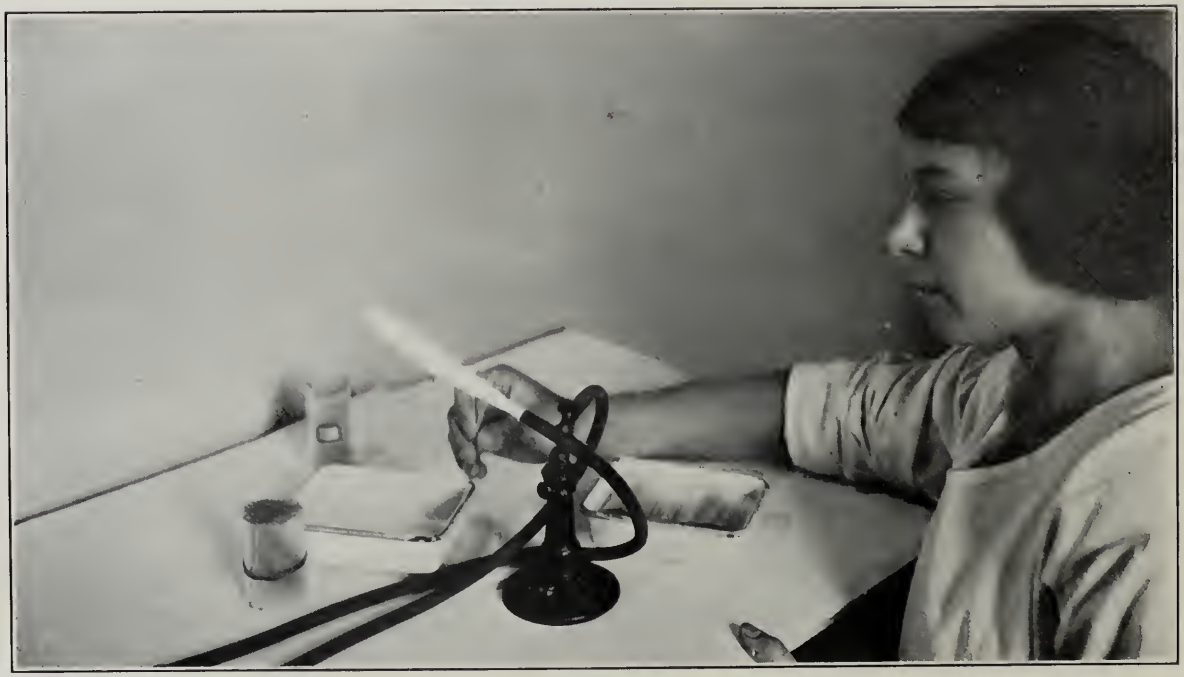
From this short sketch we see, as we said, that there are many factors to consider before we can draw any deductions as to the size and character of any virus that may come through any filter. There is no question that size is a most important factor, yet organisms larger than the estimated size of the pores of the filter may appear in the filtrate. This may be due to their plastic ability to mold themselves through spaces otherwise too small for them to pass through.

Recent workers stress the similarity of the virus diseases, but this seems easy to overstress. We are sure that among these sixty or more viruses listed by Rivers, there is more diversity than similarity. In this connection it is interesting to recall that scarlet fever, which was early put with the virus diseases, is now placed definitely with the streptococcal diseases, and the virus of pleuro-pneumonia of cattle is just within the limits of visibility. Then the kinds of tissue these viruses attack and the whole course of the infection caused by some are completely different from the mode of attack and symptoms produced by others. Thus smallpox virus attacks chiefly the skin and mucous membranes, rabies virus chiefly the nerve tissue; some, like smallpox, are extremely contagious, others, like rabies and yellow fever, spread only by special methods of inoculation.

One of the first diseases, if not the first, shown to be due to a filterable virus is foot-and-mouth disease.

Any one traveling by automobile through California or Texas during the summer of 1924 would not need

Preparation of bovine vaccine for use against Smallpox. Grinding the pulp



Sealing tubes of bovine vaccine, for use against smallpox

to ask "What is foot and mouth disease." They would know by some disagreeable experiences that it is a highly infectious disease of cattle and other cloven-footed animals, and that in order to help stamp it out disinfection is practised on a large scale, including the spraying of automobiles, camp outfits and other contents of cars. In addition, they would find that embargoes are laid on anything suspected of carrying the infection into or out of the States infected.

Though it seldom attacks man and it can quickly be controlled in animals, as the United States Department of Agriculture reports show, wild rumors of the extent of and danger from the disease caused much fear and even hysteria among citizens and travelers. Mohler [2] says that economic loss to the State of California was about $7,000,000. Rumors of business failures were greatly exaggerated.

The origin of this 1924 California outbreak was traced to garbage from the Mare Island navy yard. Presumably the virus was in some of the meat obtained from foreign ports. This garbage was fed to a certain lot of hogs, who came down with such a light attack of the disease that it was unrecognized. The hogs were sold into the county, where immediately afterward the disease began to appear among the cattle.

There is not a high mortality among the animals affected, but they may be sick for some time. They lose much flesh and weight, and their milk dries up. The disease begins with fever, then little water blisters appear chiefly on the mucous membrane of the

[2] United States Department of Agriculture, Circular 400, 1926, p. 60.

mouth, and on the skin between the toes and above the hoofs. These rupture and form erosions and ulcerations, which finally crust over and heal. The animal seems to suffer pain and is lame while the disease is in progress.

Only a few scattered epidemics have occurred in this country, but the disease is common in Europe and in some parts of Asia and South America. It has never appeared in Australia or New Zealand.

That the virus is filterable was discovered accidentally by Loeffler and Frosch in 1897. In order to get from the vesicles a clear serum freed from cell detritus they filtered it through a Berkefield filter. What was their surprise to find that this clear filtrate, when injected into cattle, caused the disease as readily as did the unfiltered virus. They ruled out the possibility of the filtrate containing only a toxin by making very high dilutions and injecting successive animals. No one has yet been able to cultivate this virus, at least in such a way that others can corroborate the work.

The second filterable virus discovered is the one that causes mosaic disease of tobacco. Beijerinck made the discovery in 1899. It has since been found that the whole group of the mosaic diseases of plants are caused by filterable viruses. Just how closely they are related is not yet determined. They usually affect the plant first by causing irregular blanching or yellowing of the part attacked. This mottling may form a sort of mosaic pattern, hence the name mosaic disease. In certain of the diseases galls or enlargement of infected areas may be produced. Certain unknown bodies included in the cells of the plant have been described,

which are thought by some to be the adult stage of the inciting agent, but this has not been proved. Then in the more poisonous types of virus groups of the plant cells die. While many of the virus diseases of plants cause heavy losses in commerce, others run a comparatively mild course and do little damage. Kunkel [3] calls attention to the fact that a mild mosaic disease has increased the value of certain plants by adding a variegated beauty to their foliage or flowers. Thus the yellow leaves of certain abutilons and the variegated flowers of some tulips are caused each by a mosaic virus.

Diseased plants sometimes recover, and resistant varieties may be cultivated. Insects carry the virus. Spraying helps a little in their control. The best method to do away with this pest, however, is to plant disease-resistant varieties.

The next filterable virus demonstrated is a very important one to man. It is the one causing that former dread of the tropics, yellow fever. It is no longer a scourge since the epoch-making work of the American commission in the Panama Zone not only demonstrated so tellingly that its cause is a filterable virus, but showed how it is conveyed and how it may be controlled.

The undiluted serum from cases of this disease was shown by the commission in 1901 to contain a virus that would pass through stone and porcelain filters. The clear filtrate obtained was found to be infectious for human beings who volunteered to allow themselves to be used for test injections, since none of the lower animals were known to be susceptible to this disease.

What bravery! especially as there was no known cure

[3] In "Filterable Viruses." Ed. by Rivers—already quoted.

for the disease. Some of them lost their lives from yellow fever in these telling demonstrations. That was a thrilling piece of work they did. They further proved that a certain kind of mosquito carried the virus, and protection from it meant no yellow fever.

The wonderful improvement in living conditions as a result of these demonstrations and the practical application by Gorgas in the Panama Canal Zone proved brilliantly the enormous value to mankind of the work and sacrifice of these investigators. The Canal Zone was formerly a notorious yellow fever and malarial district. The French commission had given up work there because of the loss by death of 50,000 or more workmen, chiefly through these two diseases. The place was called the white man's grave. Then when the American commission found out for certain that special mosquitos are the dangerous transmitters of yellow fever, as another type of mosquito had been shown to be for the malarial microbes, ways of protection from these pests were soon devised; and the practical application of them by Gorgas and Guiteras was so successful that life and work there were at last made possible. The birthplace of Walter Reed, the head of the American commission, is being made a national monument in recognition of this conquest of yellow fever. It must stand as a tribute to all who took part in this monumental work, especially to Lazear, who died, and also to Carroll and Agremonte, the other members of this memorable commission, and to Moran and Kissinger, who also volunteered for the service of humanity—to all of these it must stand as a memorial.

A non-fatal but temporarily disabling disease resembling yellow fever somewhat is dengue or breakbone

fever. This was shown by Craig and Washburn to be filterable and to be carried by the same kind of mosquito as that carrying yellow fever. Last summer quite a severe and extensive epidemic of this disease broke out in Greece, which interfered with business and travel.

The next filterable virus found is the one causing a disease which might be said to be spread chiefly through the sentimentality of people. This is that much discussed but little understood disease called rabies or hydrophobia.

The dog is the animal in which this virus is most commonly found. Though other animals are susceptible to it, the dog is the practical carrier. We have long made this cry our slogan for the prevention of rabies, "No stray dogs, no rabies," but we still have stray dogs even in the most civilized countries, and we still have rabies.

Indeed, some of the wealthiest (unfortunately wealth does not always mean intelligence) communities seem to have the most sentimentalists, who believe that nothing should interfere with the liberty—should say, liberties—of that noble friend of man, the dog. This would be more reasonable if there were not so many dogs throughout the land that run at their own sweet will, and if they happen to have been bitten by a rabid animal, they in turn may bite man, dogs or other animals.

Many people say, "Why all this hue and cry about rabies?" We have seen a number of persons bitten by dogs, even by dogs said to be mad, and these persons, though they had no treatment, did not come down with rabies. We answer, "It is true that cases of hu-

man rabies rather seldom occur after the bite of a dog." This is due chiefly to two reasons.

First, only a small percentage of biting dogs are mad; though in the last two years this percentage has increased materially. Thus, about one half of the 942 dead dogs sent to our Health Department laboratories during 1927 for diagnosis gave evidence of having died of rabies. Two years ago only eighty-five of the 360 dogs sent to us had died of rabies—a 100 per cent. increase of the proportion of dogs mad, and 500 per cent. of the actual number of mad dogs sent to us for diagnosis. Of course, there are many non-suspected biting dogs that do not reach our laboratories, so the total percentage of rabid dogs among biting dogs still remains small.

The second reason why we see so few human rabies cases after a dog bite is that human beings are not very susceptible to rabies, and so only a comparatively few come down with the disease after a mad-dog bite, even if they take no treatment. It is estimated that on an average only about 10 per cent. of humans bitten by mad dogs and untreated develop rabies. This is not a very great risk to incur. Of course, this is only an average percentage. Those people develop the disease more readily who are bitten on parts near the brain—that is, about the head—especially the face, and on other parts well supplied with nerves, such as the tips of the fingers. On the other hand, we know that people who take the Pasteur preventive vaccine immediately after having been bitten by a mad dog run on the average only a 0.1 per cent. risk of developing the disease. In other words, if you don't take the specific treatment you run 100 times a greater

chance of having rabies than if you do. It's up to you to choose. Most people choose to take the treatment.

For, once a case begins to show symptoms, we see a sight that we may never forget. The convulsed victims present a terrible appearance. At this stage there is no known cure for the disease. The same fatal outcome occurs in dogs, so that those who truly love mankind and dogs and are informed are eager to stop the disease.

The virus was shown by Remlenger, in 1903, to pass readily through the finest filters. The virus from the salivary glands is more filterable than that from the brain.

The virus, after it enters a wound, passes up the nerves and multiplies exceedingly in the central nervous system. It also develops in the large nerve cells of the salivary glands. It may be demonstrated in the sputum of infected animals before any symptoms of rabies appear.

As soon as the virus reaches the brain peculiar bodies appear in the large nerve cells. These cell inclusions are commonly called "Negri bodies," after the one who first published their discovery. One of the authors discovered these bodies at the same time independently and with Lowden made extensive studies of their nature. The conclusion was reached that these bodies are one form of the specific cause of rabies, and Williams gave them a scientific name which is long enough to forget. The practical point about these bodies is that they are diagnostic of rabies. We have developed a quick method of demonstrating them which is now used in all the laboratories of the world. These bodies only show well by this method if the brain is fresh;

therefore, an animal suspected of having died of rabies must be taken to the laboratory immediately if the best results are to be obtained.

All of the anxiety due to the fear of rabies, and all of the discomfort and inconvenience in taking the many injections of the otherwise wonderful Pasteur vaccine treatment, may be avoided by the simple and rational expedient of having no stray dogs. Cats are usually not dangerous, because while they are very susceptible to rabies they nearly always have what is called dumb rabies; that is, they become gradually paralyzed and die without biting any one. So, while we should be on our guard against the occasional cat that may develop the furious form of rabies, our slogan, which we reiterate, "No stray dogs, no rabies," is true in practice.

The recent increase in rabies in New York City and in many parts of the United States is due chiefly to the fact that people have been too sentimental or too careless to check the wanderings at large of their canine friends. They don't seem to realize that they confer a benefit on all dogs, as well as on people, by keeping their own dogs under control.

Now we come to a virus that causes a disease dreaded because it so often leaves its victim lame for life. This is the virus causing infantile paralysis or poliomyelitis. The word infantile tells the story of where we find the greatest number of affected ones. Again we have a mystery that, notwithstanding innumerable investigations, remains unsolved. We don't even know how wide-spread the virus is. An occasional case occurs in almost any part of the country, a few more in the temperate zones, and then a small epidemic breaks out like the one around Boston in 1928.

People can't be very susceptible to this disease because there are so comparatively few cases. Even in the largest epidemics only about 4 per cent. of the population are attacked. Over 80 per cent. of these have been children. More grown-up people are affected in the country districts. This is probably due to the fact that city people, as a whole, especially among the poorer classes, when infected, get the disease lightly if at all because they are a little immune. Moreover, they may not have the lasting lameness which so often follows the paralysis that develops in severe cases. With proper treatment much lameness can be avoided.

Human convalescent serum is supposed to have an inhibiting effect if given early enough. But early diagnoses are difficult to make, and not all cases respond. Many research workers are intensely studying this disease now. Due to the interest and generosity of Mr. Jeremiah Milbank, a large fund makes possible these studies in different parts of the country. This virus does not pass through our fine filters readily.

Another of these less readily filterable viruses is that causing measles. Yes, this disease so common, so definitely characteristic with its regular invasive period, its well-marked symptoms and its practically lasting immunity, must still be classed with those of unknown etiology, though a few investigators have tried to make us believe otherwise. One of these ubiquitous streptococci has been brought forward as the culprit, but we haven't yet been able to find enough evidence to convict it.

We are just through another measles year in New York City. What does "measles year" mean? you may ask. It means that every other year for the past ten

years we have had tens of thousands of cases of measles in this city, while during the alternate years there have been only thousands.

This seasonal variation, as some of you know, is due to the fact that measles, being very contagious, once started, spreads quickly through a community. All those who are susceptible and who come in contact with a case contract the disease in rapid succession. Then it takes about two years for a new group of children to become old enough to run about and so to increase the number of those who may communicate the disease. Of course, in different sections of the country this weeding out of susceptibles may occur more irregularly and at different year periods. Even in New York City the present regularity may not continue, but it has certainly been very marked during these last ten years.

The most important fact in relation to these susceptible cases is that in the very young children—under three years old—measles predisposes to a pneumonia which frequently ends with death. The older children do not often develop pneumonia. They pass through the disease usually with no bad effects. The chief cry, therefore, concerning measles, is "What can we do to prevent it in the very young?" Mothers exclaim, "Have you not a serum or a vaccine that can be used to prevent this disease, like the serums and vaccines that have done so much good work in the prevention of smallpox, diphtheria, lockjaw, scarlet fever, rabies and other diseases?"

We have to answer "No," we have no manufactured vaccine or serum for it. While we have no universally accepted serum stimulated in animals by the action of

a known germ to cure this disease, we have a serum that has a very marked action in preventing it. This serum is called convalescent serum. It is obtained from the blood of human beings who have recovered from measles.

The first doctor who decided to use such blood had reasoned that as those who had had an attack of measles usually never had another attack, the blood of such people should contain an antibody against the virus, and that such blood, if injected into those who were just coming down with the disease, might cure them or at least prevent them from having a severe case and getting pneumonia. So he collected what blood he could from those adults who had had measles and were willing to give their blood, and after testing it for its purity, he injected a number of patients with it. The good results were marked, but as he was unable to obtain much blood at a time he could not make a demonstration of its efficiency striking enough to cause the medical profession of the world to take notice. Occasionally following him a doctor would use what little blood he could collect and report favorably on its use. The chief trouble was the difficulty of getting enough blood of known potency to have the desired effect. Then about three year ago, one of us had a wonderful opportunity to get a large quantity of this measles convalescent blood.

Quite an extensive epidemic of measles occurred in a southern university where many of the students coming from rural districts had not had measles. These students volunteered in large numbers to give their blood during convalescence. So we sent one of our doctors down there with a trunk full of sterile bottles and other apparatus for drawing and collecting the

blood. At first only the boys were tapped, but when the girls objected that it was unfair they were included. In this way large quantities of blood were drawn at different periods of convalescence. With this we were able to find out definitely later the best time to draw the blood after the person had recovered from the disease. This time, they found, was from ten days to three months after recovery. With this blood serum we were able to treat nearly 2000 children during the measles year of 1926. The dose is from five to ten cc. And they were able to show very clearly that such blood serum had a marked effect in protecting from measles if given early enough, or in modifying the course of measles if given later. Even years after an attack of measles, a considerable amount of antibody remains in the blood, but of this the dose is at least six times as much.

The next important virus classed with the filterable ones is that destroyer of beauty, the smallpox virus. This too, like measles virus, passes with difficulty through only the coarser stone filters. And this too is a definite disease with characteristic symptoms. But it has an advantage over measles from the standpoint of studying it, in that it can infect certain lower animals with its characteristic lesions. Furthermore, we have the innocuous cowpox virus which we can study and from which we can make an efficient vaccine against the deadly smallpox virus.

Nothwithstanding all these aids to study, we still don't know what kind of a microbe the smallpox germ is. As in the diseases produced by so many of these filterable viruses, we find certain cell inclusions in the epithelial cells of the pustules formed all over the skin

of the sufferer, which some of us have thought might be the specific microbes. But we haven't been able to grow them and we haven't been able to recognize a clean-cut resemblance to any known microbes. So we call them Guaniari bodies, after the one who first wrote about them, and let them go at that.

The number of the descriptions of these forms and the long and complex life cycles given to them by some of our protozoölogists ought to make us sure of their nature; but no, we are just as uncertain as ever.

We are certain of one thing, however; that is, that the bovine vaccine is a powerful preventive of smallpox, and that those who don't use it in the countries where they are not forced to are extremely ignorant and foolish.

Among the other diseases declared to be due to filterable viruses there are not any of great general importance to man. Mumps, certain common colds, certain cases of grippe, among them, it is true, may be considered as decidedly disturbing at times, but they haven't the big importance that most of the other diseases cited have.

Then come the Rickettsia, those peculiar masses of irregular granules described first by Ricketts in typhus fever, that only gradually after long study by Wolbach and others are being accepted as probable microorganisms. They are found in Rocky Mountain spotted fever, trench fever, heart water disease and flood fever. They have all been traced through ticks, body lice or bedbugs as carriers. If these pests of the unclean can be prevented, such diseases cease. It is thought that typhus may also be carried directly. But in the World War it was practically shown that if troops were pro-

tected against vermin, neither typhus nor trench fever developed. It was not so many years ago that body lice traveled around freely over the tenants of the tenements of the world. We had typhus in New York. Then, with the disappearing of the lice, a mild type of typhus appeared, and now, with the lice gone (shall we say?), we have practically no cases of this type of disease. While we don't know the cause of typhus (we can't grow the Rickettsia bodies), it has been found that a certain bacterium belonging to the proteus group, isolated from typhus cases, agglutinates in the serum of any case that has typhus. We don't know why. It is simply used as diagnostic test.

That most perplexing of diseases known officially as epidemic encephalitis and commonly as sleeping sickness (number two) is an appropriate one to occupy the final place in this list of diseases of unknown origin. For we have no idea of its cause. Dr. Neal has recently summarized the little we know about it. The following are excerpts from her articles:[4]

The earliest recognized cases in the present outbreak of the disease now known as epidemic encephalitis were described by Cruchet, Moutier and Calmette in 1917. . . .

During the past ten years an enormous amount of work has been done by scientists in many countries to determine the cause of this disease, so protean in its clinical manifestations and so terrible in its chronic effects. . . .

The early conception that it was due to botulism or that it was an atypical form of poliomyelitis has been sufficiently disproved to be dismissed. . . .

There has been much discussion both for and against a relationship between influenza and epidemic encephalitis.

[4] Neal, "Present Status of Etiology of Epidemic Encephalitis," "Jour. of Am. Med. Assoc.," Vol. 91, p. 39, 1928.

This has been based on the more or less coincidental appearance of these two diseases in many places in the recent outbreak; on the possibility of the same relationship as to time having occurred in the past, and on the functional or organic disturbances of the central nervous system sometimes occurring with or following in the wake of attacks of clinical influenza in individuals. In view of the lack of knowledge concerning the cause of influenza, all the discussion seems to be of an academic rather than of a scientific nature. . . .

There are three theories which assume that: (1) it is a toxic disturbance of the central nervous system due to: (a) Toxins produced by organisms located probably in the respiratory or gastro-intestinal tract. (b) Toxins elaborated as the result of metabolic disturbances. (2) It is caused by cultivable bacteria. (3) It is caused by a filterable virus. . . .

The case for the toxic origin of epidemic encephalitis does not seem to rest on a very firm foundation. Many points of evidence in regard to liver lesions, for example, are not corroborated by other workers or are differently interpreted by them. Moreover, the manner in which the disease spreads in increasing numbers from place to place and since subsided strongly suggests that it is of an epidemic nature. In its epidemiologic manifestations it bears a strong resemblance to poliomyelitis and to meningococcus meningitis. Therefore, if one assumes that it is of a toxic nature, one is forced to hypothesize some infectious disease that was prevalent in different parts of the world for some years to the effects of which epidemic encephalitis was due.

The evidence in favor of a known bacterial cause is slight. That it is due to a filterable virus is considered probable, but even here the evidence is not clear cut. We are therefore forced to conclude that up to the present time the causal agent of epidemic encephalitis has not been made manifest.

## CHAPTER XX

## MAN MAKING USE OF HIS ACQUAINTANCE WITH MICROBES TO PROTECT HIMSELF

A SUMMARY

Clean milk—Clean water—Clean food in general—Protection against disease germs and disease carriers.

In the foregoing chapters we have attempted to give as clear an idea as possible in such a small compass of how microbes came to be known, of how they live, of their helpful and harmful effects on man and of how man has learned in some measure to control and use them.

As we have intimated, long before microbes were discovered man tried to make his life safer and more comfortable by employing certain methods that, unknown to him, depended upon the control or use of microbic growth for their successful working. The origins of many of these early uses are lost in antiquity.

The drying, pickling and freezing of meats and fish, the souring of milk, the making of wines, the retting of flax, and so on, all began to be used so early that their beginnings are mixed up with myth and tradition. Bacchus the Greek god of wine, Osiris the Egyptian god of brewing, and various other gods are illustrations of the kind of explanations given of the originators of many customs.

Even after some definite knowledge had been ob-

tained concerning microbes and their relation to disease, the great majority of physicians and sanitarians put emphasis on things which had little importance so far as the development of communicable diseases was concerned. The atmosphere was thought to have much to do with conveying the disease-producing germs, and filth was believed to be the frequent soil for their propagation.

One of the writers remembers as a student at Columbia University hearing Professor Chandler giving an illustrated lecture on sanitation. A series of pictures illustrated the deadly nature of sewer gas. For instance, the sewer received the throat discharges from a child sick with diphtheria. The diphtheria bacilli grew in the filth and in some remarkable way gained access to the sewer air, spread through the sewer, traveled up the house drainage pipe of a neighboring house and finally passed through a hole in the pipe into the air of a room and were there inhaled by a sleeping child. The unfortunate child contracted diphtheria and, according to Dr. Chandler, died from it. We now know that very few disease bacteria continue to thrive in sewage, and that sewer air and house drain air, though odoriferous, contain no sewage microbes, since these germs cannot escape from the fluid sewage holding them.

Until very recently it seemed almost evident that microbes of diseases like malaria traveled by the air route and infected persons passing through infected regions. Now we know that the spread of infection among human beings is due to the mosquito that becomes infected from a human case and then bites other humans and so inoculates them with the malarial germs.

Well into the present century it was believed by a number of sanitarians that many disease-producing microbes, as well as the spore-bearing bacilli, develop in the soil and in decomposing animal and vegetable matter. They thought that increased rain causes the ground water to rise to the upper contaminated surface soil so to become infected and later to infect surface water supplies, wells and springs. The same ideas caused great attention to be given to getting rid of nuisances and filth in time of threatened epidemics. Now we know that healthy human carriers of the germs of typhoid fever, cholera, diphtheria, scarlet fever and many other communicable diseases were the usual spreaders of infection, and that the soil and dirt were not in themselves carriers except temporarily, as they were contaminated by careless or uncontrolled human or other animal carriers.

It takes a great deal of good judgment and knowledge properly to evaluate the importance of cleanliness as a measure of insuring health. No intelligent person would consider the littering up of the streets with loose newspapers and discarded banana skins as a serious menace to health, while all would agree that the consumptive that expectorates freely on the pavements of the street is a menace.

Milk is par excellence a food material suitable for the growth and multiplication of disease microbes, especially the typhoid and scarlet fever germs. Occasionally in custards, jellies, fresh meats and canned goods that are not protected certain pathogenic varieties of germs may continue to grow and increase, but in materials containing insufficient or inadequate food elements for these microbes, such as water, dust, decomposing leaves

and the like, they may exist for a while if deposited upon them, but they do not multiply and after a time they die. The fact that microbes are invisible to unaided eyesight makes it impossible for the ordinary observer to be sure when and where infection may lurk, so that the endeavor to secure cleanliness in the person, in the home and in the town or city not only aids happiness but also health, because in removing much harmless dirt we also frequently remove dangerous microbes; in removing broken crockery, tins and any receptacle that may catch the rain-water, we prevent breeding places for mosquitos, and in keeping our kitchens and households clean we prevent the development of flies and other insects which may become carriers of disease microbes.

Thus cleanliness is a great safeguard, not so much because it removes breeding-places for microbes, but because in disposing of the waste we also remove any human disease germs which may have been added to it and make it impossible for the development of insects which may mechanically or as intermediate hosts spread infection.

The attention of those fighting communicable diseases has been more and more focused on the location of the disease in human beings and therefore the probable path of exit of the germs, the length of time during convalescence the germs persist and may be communicated to others, the existence of healthy carriers of the disease-producing microbes and the duration of the resistance of the microbes to drying, sunlight, heat and chemicals. The germs attacking the food-producing or man-serving animals have been investigated in the same way both with the idea of pre-

serving the animals for man's uses and of making life more desirable for themselves. In the few instances in which animals transfer communicable diseases to man the methods by which the infection is communicated have been especially carefully investigated.

The water, milk and food supplies have been studied so as to apply methods of preventing them from becoming infected with dangerous germs, or, when that has seemed to be impossible, to use methods that destroy the infection by heat or disinfectants.

The progress made toward victory by man against the individual microbes already described is well illustrated by his fight against typhoid fever. The discovery of the typhoid bacillus as the cause of typhoid fever was in our hands the key to unlock the mystery of its transmission. Examination of the stools of patients sick with disease proved that the typhoid germs passed with the bowel movements. Then came the surprising discovery that about 2 per cent. of the persons suffering from the disease contract a chronic typhoid germ infection of the gall bladder which makes these persons as dangerous as are typhoid fever patients. These facts brought about revolutionary changes in our preventive measures to render water, milk and other food products safe. It was found that any typhoid bacilli gaining access to the milk actually multiply in it, while those contaminating the water supply not only do not multiply in the water but gradually die. The interesting fact was noted that their period of existence is only a few days in polluted waters, but several weeks in otherwise pure water. The polluted waters contain substances inimicable to the germs.

Let us look for a moment first at the present sani-

tary methods of handling the milk situation. Raw milk is now allowed to be sold in cities only when it is produced under almost ideal conditions. Those who wish to be employed in the dairy who are to have any contact with the milk are first questioned as to whether they have ever had typhoid fever. If the answer is "yes" they are not accepted. If they are accepted, they submit to blood tests which will generally indicate whether they are typhoid carriers or not. To make assurance doubly sure, their stools are subjected to bacterial tests. The employees are also frequently examined by physicians as an additional safeguard. These conditions are so difficult to carry out that the bulk of the milk consumed in cities and towns and much of it in the country and even on farms is now heated sufficiently to kill the typhoid bacilli before it is consumed. An immense amount of investigation and experimentation has been done to establish rules for pasteurization. Since milk is one of the best of foods for all ages, it was desirable not to injure the food value, taste or appearance of the milk. Otherwise it would not be as nutritious or tasty. It was found that temperatures above 145° F. injured the little fat globules encased in the protein films, so that cream did not rise as well or seem as thick. Fortunately, it developed that an exposure of the bacteria in the milk for twenty minutes to a temperature of 140° F. was sufficient to kill all disease bacteria apt to be in milk. There remained then to study the pasteurizing machines in use to discover what were the irregularities in the rate of flow and in the temperature in different parts of the machines and to what degree these could be remedied. It was found that if we placed the minimum required temperature for holding the milk

at 143.5 and the time at thirty minutes we had a sufficient margin of safety. A code has just been adopted by the Federal Public Health Service, which will be submitted to the different States and cities, which if adopted and carried out will make pasteurized milk practically safe.

The next great source of human infection was the typhoid bacilli carried to man by means of the water he drank. Until recent times the water of streams and of shallow wells was consumed thoughtlessly by the great majority of people. In populated districts the result was disastrous. The typhoid patient or the innocent typhoid bacillus carrier, knowing nothing about the danger to others, deposited his infected fecal matter in some privy on the side of a brook or near a well. Some of this would leak into the brook or well, or perhaps a heavy shower would develop and the stream would rise, flow into the privy and sweep out the typhoid bacillus infected material into the brook and so into the reservoir, and from there finally to the city water main and to the people drinking the water.

The first attempt to remedy these evils was through filtration of the cities' water supply and the removal of privies from dangerous places. These improvements made a remarkable difference in the typhoid toll. Filtration plants, however, are costly and need expert operation. They were not practical for small places. Even in the plants of large cities at times something went wrong with the filter plants and sewage escaped into the drinking-water. Fortunately, it was then discovered that a small amount of chlorine could safely be added to the water. When the water was collected from the country-side or from the mountains and had little or-

ganic matter, even one part in a million of chlorine would kill any typhoid or other germs which are liable to cause human disease. When the water was more turbid or contained organic matter from more densely inhabited regions as much as one part in a hundred thousand became necessary. Within a few hours after its use the chlorine becomes united to the organic matter, including any microbes, and makes compounds which are absolutely harmless. At the present time fully 80 per cent. of all the water consumed in towns and cities has chlorine added. When the larger amounts are put in there is sometimes a rather disagreeable taste, but otherwise the water is harmless.

The third means by which typhoid fever is spread beyond the confines of a family where a typhoid case exists is through shellfish. Oysters naturally grow on the shores of the ocean or in the bays in which the water is not quite as salt. The bays are far more convenient for the cultivation of oysters. In time cities encroach on these bays, and the oystermen feel that as they were there first it is not their business to move but it is the business of the city to take care of its waste. This brings about frequently a condition in which oysters are being raised in polluted waters, and if they become infected they remain so for a number of weeks. When the oysters begin to die they form a splendid soil for the growth of the typhoid bacillus. Until the health authorities of the Federal Government and of States and cities took over the control of the osyter industry quite a number of outbreaks due to oysters developed. A few years ago in New York City hundreds of persons developed the disease before it was traced to the oysters. At the present time the safeguards are

so great that there is very little danger. Shellfish are safer during the cold seasons of the year, because when the water becomes cold they hibernate and during this period they drink almost no water, so that even when they lie in polluted water they are comparatively safe. Oysters when fried or stewed are safe, as the heat is sufficient to destroy the typhoid bacilli.

Owing to these safeguards which have been thrown about our milk and our water and our shellfish, there is very little danger of contracting typhoid fever in cities where the health authorities are active, except for the occasional careless carrier that may elude their vigilance. This has brought about the fact that the development of typhoid fever in the last fifty years has decreased one fifteenth of what it formerly was. When the occasional case or carrier does occur the members of the household must use extreme precautions to prevent the contracting of infection through personal contact. Hands and utensils must be kept scrupulously clean, and all fecal and other discharges must be carefully removed and sterilized.

When the writers graduated from their medical schools, the wards of the hospitals were filled in the late summer and autumn with typhoid patients. Now with the control that man exercises over the transmission of their cause, these cases are decidedly rarer and students eagerly seek to examine and study any cases that occur. There are times and places where good control of food and water is impossible. During wars, for instance, the troops frequently must live under most unsanitary conditions. Until the most recent wars, the loss from typhoid fever and dysentery was almost as great as from injuries. Again, in isolated country places

Counting colonies grown from dilutions of samples of the city's milk supply

Corner of laboratory for bacteriological examination of milk

One of a committee of famous public health officers who themselves carried through extensive experiments to test the practical efficacy of pasteurization upon poisonous microbes that might find their way into unprotected milk

and in uncivilized countries one is in danger of infection. For these conditions we resort to inoculation with typhoid vaccine, which is a suspension of dead typhoid bacilli. This gives great but not absolute protection for two years.

Somewhat similar means have cut down the distressing summer mortality from the diarrheal diseases of infants which we have learned were partly due to microbes. For many years the children and especially the infants in cities suffered from serious attacks of diarrhea whenever the heat and humidity were great. Sometimes during a very hot spell many hundreds of children and at times even thousands would die from the effects of such diarrheas.

Nearly thirty years ago while we were experimenting upon different diets for kittens, we found that those fed on raw milk developed diarrhea from it. In examining the milk we found it teeming with bacteria. This led to an investigation and we found that the milk coming in on short hauls was delivered warm into freight cars and there remained for hours before being shipped to the city. No ice was applied. The production of milk at that time was not under the safeguards now employed. The knowledge gained by this investigation started us to make a definite study as to what relation the milk bacteria and the changes caused by their growth in the milk had to the diarrheas of infants. The next summer, through a number of physicians, we selected some hundreds of infants who were less than six months of age. Those who were fed by their mothers were left on the mother's milk; those who were fed on condensed milk were left on that food. Those, and these were the majority, that were fed on store milk were divided into

three groups. One was left on the store milk, one was given good pasteurized milk and the rest were given the very best of raw milk, which we now call certified milk. These infants were watched by physicians and nurses during the summer. When an infant became ill it was taken off the milk and given the proper treatment. It was found that the babies on the breast milk thrived the best of all; then those on the clean raw milk and on the good pasteurized milk. Far behind them came the babies fed on the loose grocery milk. This last milk on examination was found to have millions of bacteria in each teaspoonful before it was heated at the homes. This showed that the cheap store milk teeming with bacteria had developed sufficient changes because of the bacteria to be harmful to infants and to cause diarrhea. On older children it had little or no harmful effect. The same experiment was tried again in the winter months, and to our surprise there was not a great difference between the results of feeding infants the different kinds of milk. The breast milk still gave the best results and the loose grocery milk somewhat poorer results, but very little diarrhea developed. When this investigation was completed we consulted with Dr. Holt, the famous pediatrician. After studying the material together we came to the conclusion that diarrheas in summer were due to several things. First, even cow's milk modified is not the equal of human milk as food for human infants. Second, cow's milk which has become contaminated with an excessive bacterial growth is distinctly harmful. Third, that careful supervision by a physician or nurse of the cleanliness of the baby, of the proper clothing of the baby, of the proper ventilation of the baby's room and the proper care of a baby dur-

ing an attack of diarrhea was of great value. These investigations were really the starting point for the modern control of the milk supply. With the development of baby health stations, the production of clean, well cared for milk, and the education of parents as well as physicians, severe summer diarrheas have practically ceased to develop. Instead of the mortality in summer mounting during the hot months owing to the deaths of little children, the mortality remains low and the intestinal diseases are a minor factor. The bacteria were shown to be the exciting factor of the diarrhea, but they were aided by the lowered resistance brought about by the summer heat, by improper food and improper care.

The question of tuberculosis in infants due to milk has received much attention. Tuberculosis is a prevalent disease among not only human beings but among cattle. It is now known that the tubercle bacilli in cow's milk produce infection in little children which may be fatal in character. In older children who have some immunity the bacilli are less deadly and only make local lesions. In adults they are practically harmless. We are able to prevent the spread of tuberculosis in infants and young children either by pasteurizing the milk or by freeing the herds of infected cattle discovered by means of the tuberculin test. This test is used in two ways. In one way an injection is made under the skin of the animal with a small amount of tuberculin. The temperature of an infected cow begins to rise at about the twelfth hour and reaches its height four to six hours later. It then slowly subsides. This infected cow is removed from the herd. The other test is what we call the intracutaneous test. A tiny amount of tuberculin

is injected through a hypodermic needle into the skin or it is rubbed into a scratch made along the skin. In about 12 to 24 hours a papular elevation develops with redness extending about one third of an inch about the point of injection or on each side of the scratch. This positive reaction condemns the cow. When either of these precautionary measures is taken, milk is safe for use by infants and children. The advantage of using the tuberculin test is that by weeding out diseased animals the other cattle are protected from infection.

Besides tuberculosis and typhoid fever, that bad condition known as septic sore throat and its frequent companion—scarlet fever—are transmitted through milk. These conditions are both due to streptococci, which have been described in an earlier chapter. The streptococci are conveyed to the cow by a man or woman who milks a cow while suffering either from a light attack of scarlet fever or septic sore throat, or after becoming a carrier of the germs. The hands previously infected from the mouth secretions infect the cow's teats. A little inflammation develops in the lining membrane of the teats or udder. Since milk is a good food for streptococci, they multiply greatly in it and so are conveyed to those who consume it. There have been outbreaks of septic sore throat and scarlet fever in which as many as 2000 people have been infected.

Pasteurization is the only certain protection, but careful observation of the milkers and thorough daily inspection of the herd of cows will almost surely prevent infection being passed through the raw milk.

Diphtheria is also passed through milk, but much less frequently. This generally arises from some ignorant person who is a diphtheria carrier using a dipper or

some other utensil to take a sip of milk and then putting the contaminated ladle back again into the milk to cause infection. The streptococci may contaminate the milk in the same way. All these bacteria except the tubercle bacilli actually grow in the milk when they have once entered it.

The tubercle bacillus has been fought in still other ways than through safeguarding the milk supply. Wherever man has been massed, tuberculosis has been present. Until recently it produced more illness and deaths than any other infection, and is probably now only second in its occurrence to pneumonia in the temperate zone and to malaria in the tropical zone. Both animals and man are almost free of tuberculosis when living a free life in the open air, but when confined in crowded, closed-in quarters the germ develops freely in them. In fact, a very great percentage of all persons dying in civilized communities show evidence of having at some time been infected with the tubercle bacillus.

For nearly seventy years there has been an almost continuous decline in the amount of serious tuberculosis. Thus in Scotland the death-rate in 1881 was 17 per cent. less than the average death-rate of the ten years before, of 1891 it was 21 per cent. less, in 1901 it dropped 9 per cent., in 1911 it dropped 21 per cent. and in 1921 there was a drop of 31 per cent. as compared with the previous ten years.

In the United States in 1901 the death-rate was 195.2 per 100,000, in 1911 it was 164 and in 1920 it was only 112. During the last fifty years the death-rate from all causes has been reduced less than one half, while that from tuberculosis has been reduced more than two thirds. There is still, however, much

disease and a staggering number of deaths. Thus, in New York State during 1925, about 10,000 persons died from tuberculosis. There would have been, however, more than 40,000 deaths if the amount of tuberculosis had remained the same as fifty years ago, the death-rate having been reduced in New York City from 400 per 100,000 to 100 in that time. This great improvement has taken place, as stated in Chapter XIII, because of the better living conditions, the greater knowledge of the sources of infection and means of preventing infection, the removal of many of the sick to sanatoria, and the tendency of the tubercle bacillus to decrease in virulence both because it grows more slowly in the average case and because the most severe cases are chiefly in hospitals and so the infection passes usually from the milder cases to new individuals. Whether or not we obtain a successful vaccine such as Calmette in his attenuated bacillus hopes to have is still doubtful. There is no doubt, however, that if we keep up our attack on the tubercle bacillus in the same vigorous way that we have been doing, tuberculosis in the next twenty years will be one of the less important diseases.

Man's success against the microbes in the tropics is, on the whole, a striking one. There are important diseases such as pneumonia, typhoid fever, smallpox and measles which develop in all parts of the world. These occur in the tropics as elsewhere and we have already considered them. The essentially tropical diseases are almost wholly those in which insects or vermin act as carriers of infection from the sick to the well. The tropical climate, with its warmth, humidity and luxuriant vegetation, makes life easy for the insects and

makes it difficult for man to eradicate them. These conditions to some degree pertain in subtropical and temperate regions, where the winter is not cold enough to destroy semi-tropical vegetation or the dangerous insects. The infections which have occurred in tropical or subtropical regions are capable of persisting in those who remove to colder climates. They may occur in other countries in the season when the conditions approach those of the tropics. The conditions under which people live have a great influence in making them more or less liable to infection.

Most of the tropical diseases due to microbes are transferred by insects. Yellow fever, once the dreaded foe of the white man, is transmitted by the mosquito. The brilliant and heroic investigations of the United States army commission already described led to an understanding of the conditions which made it possible for the mosquito to breed and for the sick man to infect the mosquito and for the mosquito to infect man. Once understood, it was simply a challenge to the human race to exterminate these conditions. Yellow fever is now endemic only in certain parts of Africa and in one or two countries of South America. The human race has practically conquered the yellow fever microbe and does not need to consider it in planning trips or places of abode.

Another dreaded disease peculiar to the tropics is African sleeping sickness. This as already stated is due to a protozoan called a trypanosome, which is transmitted chiefly by a biting fly belonging to the genus Glossina—the tse-tse fly. This disease is also largely limited to Africa. The mature flies lay their eggs in the shade of trees and small shrubs near the

water. These are just where people are apt to pitch their habitations or make their resting-places and so make it easy for the sick to infect the flies and for the healthy to receive infection. The flies generally bite only in the daytime. It is much more difficult to prevent the spread of sleeping sickness than of yellow fever. The yellow-fever-carrying mosquito develops in little pools of water, such as in cisterns, in broken crockery about the house, etc. It is easy to remove all such possible places for holding water. The sleeping-sickness-carrying fly develops in the tropics wherever shade and moisture exist. Furthermore, there are animals as well as human beings which can be infected with the species of trypanosomes which infect man. Up to the present time only fair progress has been made. We try to isolate the patients so as to treat them with curative drugs and to remove them from the presence of flies. As far as possible we destroy the animals which act as hosts for the parasites and we cut down the trees at stopping-places which are found to shelter the flies. Immigration from and visits to infected areas are limited as much as possible.

Malaria can properly be considered a tropical disease, because while it develops also in subtropical and temperate climates, it is most severe and wide-spread in the tropics. This disease is carried by a mosquito of a different species from that carrying the yellow fever germ. It is called anopheles. These are not home-loving mosquitos. They develop in the water of swamps, puddles, shores of stagnant streams. With the rainy season they increase enormously. When the rainy season is over, the shallow collections of water dry up as the larvæ disappear, while many of the adults keep alive

in shady places and wait for the next rainy season. Some of these mosquitos breed in tree holes or in the water contained in bamboo joints.

The outdoor mosquitos only invade human habitations when they are near their breeding-places. Workmen camping in open ranches or people traveling on rivers are the most exposed. It is easy to see the difficulties which we meet with in trying to eliminate malaria. In a settled community it is possible, but in the sparsely populated regions it is impossible.

We attack the problem in two ways. We use daily small doses of quinine as a preventive of infection and larger doses to cure the case; or, if this is impossible, to eliminate the parasite from the blood and to prevent infection of mosquitos. Wherever possible we diminish the number of mosquitos by the drainage or filling up of pools or swamps or by cutting down excessive woods. The homes of the people are built on fairly high and dry ground. The windows are screened and as far as possible infected people are isolated. Chronic cases especially are sought for, and the blood is examined for the parasite in suspected cases.

We have found an additional means of defense. Many varieties of fish eat the larvæ voraciously. These are grown in the waters where the mosquitos breed. By these various means man has made great progress in the more civilized and well-inhabited regions, but he sees the time as still far distant when he will obtain a complete victory over the malarial microbes in all parts of the world.

Our progress in attacking the microbes causing the venereal diseases is growing slowly. These ubiquitous microbes have a peculiar relation to human infections.

Except in very unusual circumstances, there is no excuse for their transmission. The one who passes the infection to another is aware of the danger, or at least should be aware, but the recipient of infection may in all innocence receive it. In spite of these facts, venereal diseases still abound and the innocent as well as the guilty suffer irreparable harm.

The American people have resolved to continue an ever-increasing battle against the microbes of gonorrheal and syphilitic infection. They believe that a courageous facing of facts and the pressing upon the minds of young men especially the harm that these infections do, not only to themselves but to innocent women and unborn children, will persuade them to take the necessary precautions. The American plan does not regard venereal disease as a punishment but as a communicable disease which must be treated not as a private matter but as one of public concern. It emphasizes the fact that the sex instinct is normal, and urges education rather than prohibition. It considers the infected as patients and not as animals. It urges that society provide opportunities for healthful recreation. It expects woman as well as man to take an active part in the campaign. The progress made is encouraging, considering the short time that the public has made organized attacks upon these diseases. The Federal Government as well as States and cities is appropriating large sums of money for the prevention of venereal diseases. Private organizations are giving active and effective help.

We may conclude our summary by a brief statement of how vaccines and serums have helped to protect man from harmful microbes. It is common knowledge

that many diseases give protection to those who have recovered from them. No one fears allowing a child who has just had measles or scarlet fever to be placed in contact with a child having one of those diseases. This protection is what we call "specific"—that is, the child which has recovered from measles is immune to measles but not to scarlet fever or any other disease. This immunity is due to two kinds of antibodies and probably also to other changes not so well recognized.

It was accidentally discovered in the early nineties that in those that had recovered from several of these diseases antibodies could be definitely found. These either neutralized the poisons of the germ, as in diphtheria or tetanus, or together with certain tissue cells of the body fought germs and destroyed them, as in the case of pneumonia. It was discovered that animals injected with these living germs or with their toxins develop one or the other of these kinds of antibodies, and that when these injections are repeated from time to time the potency of the serums gradually increases until it is so strong that it can be used in human beings, giving to them somewhat the same protection as if they had had the disease. It can be used thus either as a preventive measure or as a curative measure. We call the microbes themselves or their products vaccines, because the first vaccine was made from a calf having cowpox, and now the idea of protection gives the word its meaning rather than the animal from which it was derived.

Vaccines may be either the living attenuated virus, as the cowpox against smallpox, or larger numbers of the dead germs as in the vaccine against typhoid fever; or, instead of living or dead germs, a vaccine may con-

sist of the toxin of the germ. This latter kind can be used as a preventive in diphtheria, scarlet fever and tetanus. These toxin vaccines may be used just as developed in cultures, as in scarlet fever, or the stronger ones may be changed by the addition of formalin so that they are less irritating, as in the case of diphtheria toxoid and tetanus toxoid; or they may be made into a mixture with antitoxin—the famous toxin-antitoxin mixture, or the so-called TA. The vaccine causes the body to produce protective substances in the same way as does the disease. This is called "active immunity." It is fairly or quite permanent following the use of some vaccines and temporary from others.

Horses or other animals producing serums containing antibodies have themselves been vaccinated, but when their serum is used to protect human beings or to cure them the horse serum with its antibodies is simply transferred and diluted in the human body. This is called "passive immunity," because the body does not increase it in any way. The antibody acts essentially in the body as it would in the fluid of a flask. Unfortunately, passive immunity is not lasting, because the antibodies are a part of the blood of the horses and are to some degree alien to human beings. That is, they are foreign proteins, and any of them that are left after neutralizing or rendering harmless the toxins they have met are gradually made useless by the cells of the human body or are eliminated from the system by the urine. In doing this the human body has rendered itself sensitive to them as foreign proteins, so that a second injection is more likely to cause what we call serum sickness. This is shown usually by a skin reaction like hives and a temperature. The difference be-

tween the more lasting protection afforded by vaccination as contrasted with the shorter period from the injection of antibodies is largely due to the fact that in one case the body develops its own tissue antibodies, while in the other case it receives the tissue antibodies of another species.

Man, then, when sick uses an antiserum to help cure him; when well he uses a vaccine so he may help to establish an active lasting immunity.

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